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Cost of Sustained and Burst Swimming to Juvenile Coho Salmon (*Oncorhynchus kisutch*)

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Puckett, K. J., and L. M. Dill. 1984. Cost of sustained and burst swimming to juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 41: 1546–1551.

The relationship between oxygen consumption rate (milligrams per kilogram per hour) and sustained swimming speed (calculated from tailbeat frequency) was determined for 1.2-g juvenile coho salmon (*Oncorhynchus kisutch*) at 15°C. The data are best described by the following equation: log oxygen consumption rate = 2.2 + 0.13(body lengths·s^{-1}). This relationship is very similar to that extrapolated for sockeye salmon (*O. nerka*) of the same size, thus potentially enabling researchers to utilize the extensive sockeye data base to predict metabolic rates of coho. The oxygen consumption rate during burst swimming (9 body lengths·s^{-1}) was also determined. The burst swimming metabolic rate (38 000 mg O_2·kg^{-1}·h^{-1}) is nearly 40 times greater than the maximum sustained swimming metabolic rate.

On a déterminé la relation entre la consommation d'oxygène (milligrammes par kilogramme par heure) et la nage prolongée (évaluée d'après la fréquence des battements de la queue) chez les saumons coho (*Oncorhynchus kisutch*) juvéniles de 1,2 g à 15°C. La meilleure représentation des résultats de cette étude est l'équation log de la consommation d'oxygène = 2,2 + 0,13 (dimensions corporelles·s^{-1}). Comme cette relation se rapproche beaucoup de celle qu'on obtient par extrapolation pour le saumon nerka (*O. nerka*) de même taille, les chercheurs pourraient peut-être se servir de la banque de données très complète dont ils disposent sur ce poisson pour déterminer le métabolisme du saumon coho. On a également déterminé la consommation d'oxygène durant la nage d'accélération (9 fois les dimensions corporelles·s^{-1}). Le métabolisme durant ce type de nage (38 000 mg d'O_2·kg^{-1}·h^{-1}) est presque 40 fois supérieur à la valeur maximum mesurée durant la nage prolongée.

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The standard and active metabolic rates of fishes have been studied extensively (reviewed by Brett and Groves 1979). This accumulated knowledge and refinement of technique has enabled researchers to determine the metabolic costs associated with such ecologically relevant activities as escape from predators, prey capture, migration, and territorial defense (Feldmeth and Jenkins 1973; Brett 1983; Feldmeth 1983).

We investigated the energetics of feeding territoriality in juvenile coho salmon, *Oncorhynchus kisutch*. Our ultimate goal was to relate field swimming speeds to laboratory-determined oxygen consumption data, thus enabling us to calculate activity energy budgets that would reflect natural stream conditions. In this paper we examine the relationship between swimming speed and oxygen consumption.

Fish use streambed heterogeneities, and possibly position in relation to other fish, to their hydrodynamic advantage (Feldmeth and Jenkins 1973; Weihs 1973). Thus, in the field, and in a simple respirometer, a water velocity measurement is insufficient as an indicator of swimming speed because the exact stream flow against which the fish is working is not known. Bainbridge (1958), working with trout (*Salmo gairdneri*), dace (*Leuciscus leuciscus*), and goldfish (*Carassius auratus*), determined the relationships between tailbeat frequency, tailbeat amplitude, total fish length, and swimming speed. Several other investigators repeated this work with various species and usually found a similar relationship (Hunter and Zweifel 1971; Smit et al. 1971; Webb 1975). Tailbeat frequency is a powerful tool for quantifying swimming speeds in unrestrained fish (Feldmeth and Jenkins 1973), and was used here.

We test whether the relationship between tailbeat frequency and swimming speed in juvenile coho is similar to that described by the general equation of Bainbridge (1958). We then examine the metabolic rates associated with sustained swimming (sensu Hoar and Randall 1978) at various speeds (calculated from measured tailbeat frequencies). Finally, we present data on the metabolic cost of burst swimming to underyearling coho salmon. The ability to accelerate quickly, and to maintain high speeds for a few seconds, is a critical part of fish locomotion. Juvenile coho salmon, for example, routinely "charge" at conspecifics during territorial defense. Despite its ecological importance, the metabolic cost of burst swimming has not been empirically investigated, although it has received extensive theoretical consideration (reviewed by Webb 1975; Hoar and Randall 1978).

Materials and Methods

Speed and Tailbeat Frequency

Fish taken from the Salmon River, Langley, B.C., in May 1980 were transported to the Bamfield Marine Station. The fish

were held as a group in well water for 1 wk before experimentation. A Brett respirometer (Brett 1964, 1965) was used, since it had been specifically designed to reduce wall effects, turbulence, and eddy formations, making it possible to equate swimming speed with water velocity.

Three fish, 40-mm fork length, were tested simultaneously in the respirometer to determine the relationship between water velocity and tailbeat frequency. Water temperature in the respirometer was 13 ± 0.2°C. (Statistical limits are reported as ±1 SD unless otherwise stated.) The test was repeated using the same group of fish on five different days. Fish were placed in the respirometer and allowed to acclimate by swimming at less than 6 $cm \cdot s^{-1}$ for 12–14 h. The water velocity was then increased to 6 $cm \cdot s^{-1}$ and after 15 min the tailbeat frequency was recorded. Velocity was increased in increments of about 3 $cm \cdot s^{-1}$ every 0.5 h until the fish could no longer swim against the current.

Tailbeat frequency was recorded using an overhead video camera, and tapes were later viewed in slow or stop motion. Ten tailbeats were counted at one to six different tape locations (i.e. replicates) depending on clarity of the record. The time elapsed was noted (from a stopwatch) and tailbeat frequency was calculated from these measurements.

A few samples of tailbeat amplitude were measured from the most outside lateral position of the caudal fin on one side of the body axis to the most outside lateral position on the other side. There was no significant difference between the tailbeat amplitudes measured at the different speeds within the range we considered. The mean amplitude was 0.88 ± 0.04 cm, giving a ratio of mean amplitude to total length of 0.20. (Fork length was converted to total length using the equation of Dahlberg et al. (1968) for juvenile coho.) In all conversions of tailbeat frequency to swimming speed, amplitude was assumed to be constant.

Sustained Swimming

A different, small Brett-type respirometer was designed to measure the cost of swimming to 40- to 60-mm fish. The experimental chamber consisted of a 27-cm-long, 2.3-cm-I.D. cylindrical glass tube with wire mesh at either end. The downstream mesh was electrified and delivered a 1- to 2-V shock to the fish upon contact. A piece of black tape was wrapped around the outside of the experimental chamber to act as a visual reference for the fish.

Tygon tubing connected the experimental chamber to a 93-W centrifugal water pump and to an air-saturated water box. The pump was connected to a rheostat making it possible to alter water velocity. Temperature and oxygen probes were inserted into PVC ports in the tubing section which passed through the box. Snapping apart tubing connectors inside the box opened the system. The entire respirometer (total volume = 440 cm^3) and the water box were submerged in a large water bath for precise temperature control (15 ± 0.3°C).

Each fish was allowed to swim at a low speed overnight and then the velocity was increased to just less than 1 $L \cdot s^{-1}$ (body lengths per second, judged from tailbeat frequency) for 3 h before an experiment began. The respirometer was then closed for 30–45 min while the metabolic rate and tailbeat frequency at this speed were determined simultaneously. The respirometer was opened and flushed with air-saturated water for 15 min. Water velocity was then increased by increments of approximately 0.7 $L \cdot s^{-1}$ until no further pump output was possible, oxygen consumption being measured in 30- to 45-min periods separated by 15-min reoxygenation periods. All oxygen consumption rates represent total consumption minus a blank (oxygen depletion in the absence of a fish). Swimming activity was recorded on video tape, and the tapes were later reviewed to determine the tailbeat frequencies associated with each oxygen consumption datum. At the end of an experiment the fish was removed from the respirometer, anesthetized, weighed, and measured (fork length). Average fish size was 4.9 ± 0.18 cm (fork length) and 1.2 ± 0.14 g (wet weight). A total of 81 oxygen consumption measurements were made on 14 different fish collected in 1980 on the Salmon River.

Burst Swimming

During June and July 1981, coho fry were collected from the Salmon River and held separately in visually isolated aquaria for at least 1 wk before experimentation. The light regime was 16 h light : 8 h dark at 150 lx. The water temperature was kept at 15 ± 0.2°C. Fish ate brineshrimp three times per day (at irregular intervals) for a total ration of 3% body weight per day.

The respirometer used in the sustained swimming experiments was also used for these burst swimming experiments, but with the following alterations: (1) a peristaltic pump reduced the total respirometer volume to 240 cm^3 (exps. 1–8) or 294 cm^3 (exps. 9–19), (2) a feeding port was added to facilitate stimulus introduction, and (3) the experimental chamber was increased in length to 39 cm (exps. 1–8 (27 cm)). The potential charge distance (black tape to front of chamber: 22 cm) was the same in all experiments.

An experiment refers to a full day of measurements. The first experiments consisted of a test sequence: subsequent experiments consisted of both a test and a control sequence. Each sequence consisted of five trials. During each trial, which lasted about 0.5 h, the decreasing oxygen concentration was measured by the in-line oxygen probe whose output was recorded graphically. At the end of a trial, oxygen-saturated water was circulated through the system until saturation values were reestablished (about 15 min). In experiments 9–12, the control sequence preceded the test sequence; the sequences were later reversed when a bias was suspected.

An idealized experiment is diagrammed with hypothetical results shown on two different scales (Fig. 1). The top scale depicts the oxygen concentration measurements as recorded over time by the chart recorder. The lower scale depicts the oxygen consumption rates (minus a blank) derived from the upper scale oxygen concentration measurements. Preliminary studies indicated that blanks remained constant throughout experiments and therefore these were measured only at the end of each experiment.

The experiment began when a coho fry was anesthetized lightly with MS-222 and placed into the respirometer the night before the measurements. Once the fish appeared calm, it was fed brineshrimp and trained to avoid the electrified grid. The fish in these experiments were never starved but kept on a 3% body weight ration, even in the respirometer. The daily ration was divided into three equal portions. The first portion was given just after the lights were turned on in the morning. A second portion was delivered to the fish during the first trial of the test sequence (T1) and a third portion delivered during the first trial of the control sequence (C1). To increase the number of charges possible from one portion, small brineshrimp were used; on average, about 15 brineshrimp made up one portion.

After the fish had learned to avoid the shocking grid it was left

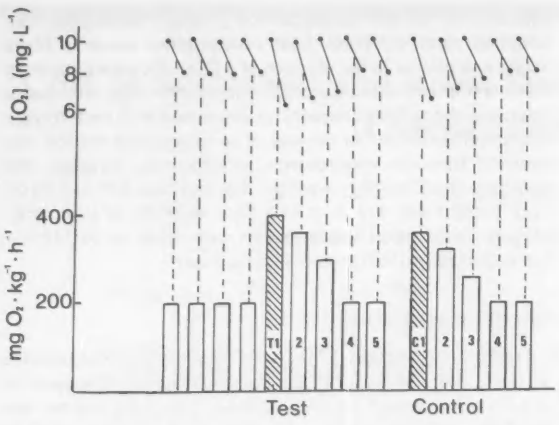

FIG. 1. Sequence of events in a hypothetical burst swimming experiment diagrammed with idealized results. Oxygen consumption rates (lower scale) were calculated from oxygen concentration data (upper scale). Test and control sequences (each consisting of five trials) are also shown, and hatched trials indicate that brineshrimp were delivered to the fish causing either burst swimming (T1) or nonburst swimming (C1).

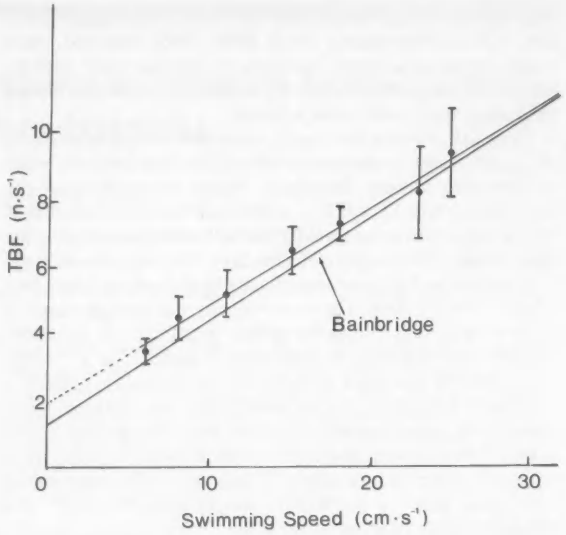

FIG. 2. Mean (±2 SD) tailbeat frequency (TBF) and swimming speed (water velocity) for three 40-mm coho. The upper line is the least squares regression ($r = 0.99$, $P < 0.01$). The lower line was calculated from Bainbridge's (1958) equation. One body length per second is approximately equal to 4.5 cm·s^{-1}.

undisturbed for 6–8 h. In the morning, the lights went on and the fish was fed. Two to four trials were then conducted to establish the baseline metabolic rate (BR) of the fish while it swam slowly behind the black tape. During T1, a single brineshrimp was forced through a syringe needle into the submerged, stoppered feeding port. The shrimp was moved by the water current into the upstream end of the experimental chamber. As the brineshrimp entered the chamber, the fish immediately became aware of its presence, charged at it, consumed it, and then returned (usually by gliding) to its former holding position behind the black tape. Once it had regained this position, other brineshrimp were introduced to the fish sequentially until all 15 shrimp had been eaten. The next four trials (2–5) reflect the oxygen consumption of the fish as it swam slowly behind the black tape (no brineshrimp offered) and were meant to measure oxygen debt caused from the burst swimming activity in T1. (All calculations of charge cost include the time between trials (used to reestablish oxygen saturation) by extending the mean oxygen consumption rate through the subsequent 15-m period.)

During C1, the fish was cued to a forthcoming feeding: 0.1 cm of freshwater, in which many brineshrimp had recently lived, was forced through a syringe needle into the feeding port. This stimulus caused the fish to move slowly to the upstream end of the experimental chamber. Immediately following the stimulus, 15 small brineshrimp were forced through the syringe needle into the feeding port of the respirometer. These tended to form a loosely defined clump and were eaten individually, or in clumps, immediately as they entered the experimental chamber. During the rest of the control sequence, the fish swam routinely behind the black tape. Thus, the behavior of the fish during the control sequence was similar in all respects, except charging, to their behavior during the test sequence. (A few charges sometimes occurred during C1 if the fish missed one of the shrimp the first time around and then charged at it as it entered the experimental chamber for the second time.) Any difference in oxygen consumption between control and test sequences can thus be attributed to the larger number of charges during T1.

At the end of the day, the fish was removed from the respirometer. After a 24-h starvation period, it was anesthetized, weighed, and measured (fork length). We completed 19 experiments using 14 different fish.

We used an overhead video camera to film fish activity and later viewed the tapes in slow motion to determine charge speed (average distance travelled over time during T1 from two experiments, $n = 25$).

Results

Speed, Tailbeat Frequency, and Sustained Swimming

We found no significant difference between the regression line fitted to our data relating swimming speed and tailbeat frequency and the curve for the same size fish derived from Bainbridge's (1958) equation (Fig. 2), supporting the application of this equation to convert tailbeat frequency to sustained swimming speed in juvenile coho salmon. The curve in Fig. 2 is only useful above 3 beats·s^{-1}. Caudal propeller efficiency apparently drops quickly at low speeds and fish switch to using their pectoral fins for locomotion (Hunter and Zweifel 1971).

The sustained swimming metabolic rate data set is described by an exponential model ($\log O_2$ consumption rate = $\log a + b$(speed)). The mean regression coefficients describing the 14 individual experiments are 2.2 ± 0.18 ($\log a$) and 0.14 ± 0.03 (b). We pooled these data (Tytler 1969; Smit et al. 1971) and calculated a mean line of best fit: $Y = 2.2 + 0.13X$ (Fig. 3).

Burst Swimming

The burst swimming (test) sequence is more costly than the control sequence (Table 1), as easily seen when these data are plotted (Fig. 4). The cost per charge was calculated as the difference between the areas under these two curves (80 mg O_2·kg^{-1}) divided by the mean net number of charges (the number of charges during T1 minus the number of charges

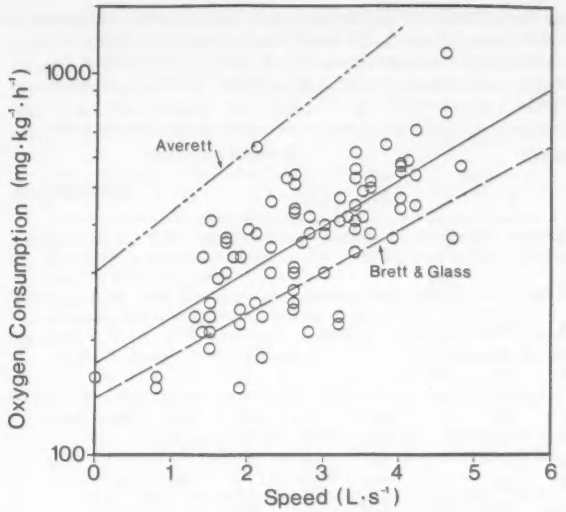

FIG. 3. Sustained swimming metabolic rates for juvenile coho from our data (circles and solid line) and those of Averett (1969) (upper broken line). Each datum represents one oxygen consumption rate determination at a given speed (calculated from tailbeat frequency). The lower broken line is an extrapolation of Brett and Glass' (1973) equation for sockeye. All regression lines are for 1.2-g fish at 14–15°C.

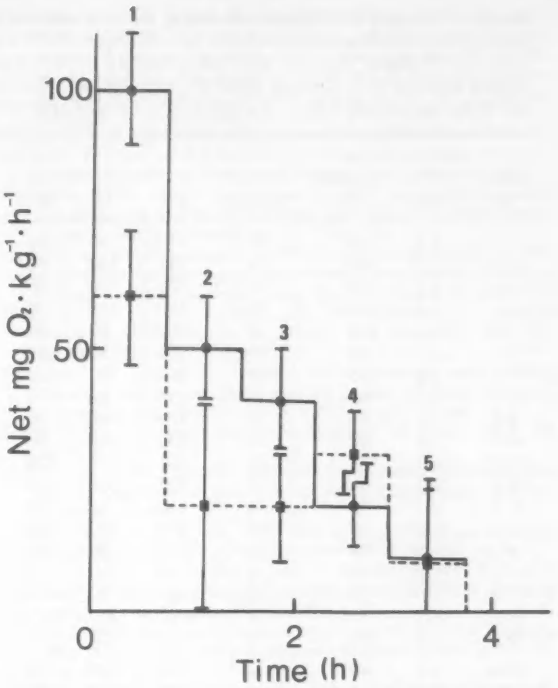

FIG. 4. Net burst oxygen consumption rates ($\bar{x} \pm 1$ SE) as a function of time from the start of the test (solid line and circles) or control (broken line and squares) sequence. Trial number is indicated above each pair of means.

during C1 (usually 1 or 2)). The average net number of charges was 14. Thus, the estimated cost per charge is 80/14 = 5.7 mg $O_2 \cdot kg^{-1}$.

The values for T1 were higher when the control sequence was run first ("C" under Exp. No., Table 1) than when it was not. This difference is significant ($t = 2.95$, $P < 0.01$). A second estimate of cost per charge of 5.0 mg $O_2 \cdot kg^{-1}$ is obtained if this "C" first group is excluded from the T1 column mean. The values for the "C" groups listed under other columns (test or control sequences) are not significantly different from the rest of the values.

The second estimate is probably the most accurate, since higher estimates may include excitement. For example, the control-first oxygen consumption rates were thought to be high even during the experiments, and this was part of the motivation for changing to the test sequence first. In general, the fish displayed considerably more excitement if they were fed many brineshrimp at once (as in C1) followed by brineshrimp fed singly (as in T1). Control-first fish tended to take longer before returning to the black tape holding position between individual shrimp feedings and to make more irregular movements. It appears that two regular 4-h feedings were sufficient to cue the fish to expect another clumped feeding in 4 h. Having the test sequence first tended to prevent any such cueing. Brett (1976) showed that fish can readily be trained to expect food and that they show an immediate metabolic rate increase under such circumstances. Before we used a fish in an experiment, it was fed three times daily but not at regular times, to avoid such problems.

Metabolic rate during a charge can be calculated from the cost per charge and the average charge duration of 0.48 ± 0.15 s as 43 000 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (estimate No. 1) or 38 000 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (estimate No. 2). The average distance travelled per charge was 18.8 ± 3.08 cm. The average speed was therefore 39 cm·s^{-1} + 4.5 cm·s^{-1} (water velocity) = 43.5 cm·s^{-1} (9 L·s^{-1}).

Discussion

Juvenile coho salmon defend feeding territories soon after emerging from the gravel streambed. The fish search from stationary swimming positions, facing upstream. Prey items from the drift float past the salmon and they dart out to pursue these prey or to fend off intruders. The metabolic cost of this activity likely influences the overall growth and fitness of salmon.

In this portion of our study on the energetics of feeding territoriality we have reported burst swimming metabolic costs and sustained swimming metabolic costs as a function of swimming speed (calculated from tailbeat frequency). The data relating swimming speed and tailbeat frequency are in good agreement with the general equation derived by Bainbridge (1958). We have used this relationship to convert both laboratory (this study) and field (Puckett and Dill 1985) measurements of tailbeat frequency to swimming speed.

The plot of oxygen consumption rate as a function of swimming speed (Fig. 3) demonstrates that there is considerable scatter in the data. Excitement (this need not be observable) could lead to an overestimate of low velocity values whereas anaerobic metabolism at medium and high velocities would underestimate these values. Juvenile coho are easily excited (Brett et al. 1958; Averett 1969) and anaerobic metabolism contributes to the energy utilized at intermediate and prolonged speeds (Beamish 1978; Wokoma and Johnston 1981; Duthie

TABLE 1. Oxygen consumption rates during and after burst swimming. The "C" under "Exp. No." indicates that the control sequence was run first; control sequences were not run until experiment 9. Experiments 1 and 2, 5 and 6, and 7 and 8 are duplicate pairs, run on the same day. "Charges" listed are those counted during the first trial of test sequence (T1) minus those counted during the first trial of the control sequence (C1). Baseline metabolic rates (BR) represent total oxygen consumption ($mg \cdot kg^{-1} \cdot h^{-1}$) before T1 or C1. All other metabolic rates represent net consumption (total metabolic rate minus BR).

Exp. No.	WT (g)	Length (mm)	No. of charges	Test sequence BR	T1	2	3	4	5	Control sequence BR	C1	2	3	4	5
1	1.1	48	14	190	90	80	40								
2	1.1	48	14	230	140	40	0								
3	1.2	49	12	220	60	90	100		−80						
4	1.2	49	10	210	140	20									
5	1.2	48	16	180	80	50	50	40	0						
6	1.2	48	17	180	80	70	40	0							
7	1.2	49	14	180	100	120	50	30	70						
8	1.2	49	14	230	80	−30									
9 C	1.1	48	15	170	90	30	20	20		120	130	60	70	40	
10 C	1.1	47	20	200	160	80	50	10		250	20	−70	−30		
11 C	1.1	47	21	120	130	100	60	50		150	70	70	0		−20
12 C	1.1	48	17	200	200	20	30	50	10	200	60	−10	50	0	
13 E	1.3	50	14	140	30	20	−40	−20	−20	120	20	20			
14 C	1.0	48	11	290	160	−10	30	−20	20	260	80	70		50	30
15 E	1.2	50	14	150	70	50	−20	0	0	150					
16 E	1.2	50	13	130	50			0	40	130	50		−10	50	
17 E	1.6	53	13	160	70	30		−20	20	170	30	50	20	30	
18 E	1.3	51	8	110	100		80	50		160	50		30	30	30
19 E	1.2	50	15	110	160		50	70		200	100	−60		10	
Mean	1.2	49	14	180	100	50	40	20	10	170	60	20	20	30	10
SD	0.12	1.47	3	50	50	40	40	30	40	50	40	60	30	20	30

1982; Jones 1982). The combined effect of these two factors would be to underestimate the slope and overestimate the intercept.

Figure 3 also compares our data with extrapolated relationships from the work of Brett and Glass (1973) on sockeye (*Oncorhynchus nerka*) and Averett (1969) on juvenile coho. Because there is no significant difference in the slope, or the *y*-intercept, between Brett's relationship and ours, we suggest that there is little difference between juvenile coho and juvenile sockeye metabolism, at least at this one fish size and water temperature. We cannot explain the discrepancy between our data and those reported by Averett (1969).

Several factors in the burst swimming experiments reduce the precision of the cost estimate, including the use of food as the charge stimulus and the aerobic measurement technique. For example, although burst swimming is anaerobic (Jones 1982), oxygen consumption was measured as the indicator of cost (see Brett 1964). Measurements of oxygen debt assumes that all lactate produced during the anaerobically powered activity is subsequently oxidized to carbon dioxide and water or converted to glucose in the presence of oxygen, but not excreted (Beamish 1978). Unfortunately, the exact fate of lactate in fishes is not known. Nevertheless, oxygen debt should be a reasonable indicator of the minimal cost of burst swimming in our experiments, since (1) salmon are not hypoxia adapted, (2) the ambient oxygen concentration was near saturation at all times, and (3) the activity was of short duration (less than 15 s total) and the oxygen measurement period was long (4 h).

The metabolic cost of burst swimming has not been determined empirically until now, but estimates based on power–performance data sets do exist (Brett 1970, 1983). These estimates are calculated by extrapolating experimentally determined relationships between swimming speed and oxygen consumption to burst swimming speeds. The estimates vary greatly and the value presented in this paper (38 000 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) is about 5–20 times greater than extrapolated values (including our own) for small coho. Fast start acceleration is thought to be very inefficient (Webb 1982) and our data support this argument.

Our estimate of the metabolic cost of burst swimming seems surprisingly high until one considers the number of charges at this rate that would be necessary to double the routine metabolic rate of a fish in the field. Suppose that a juvenile coho has an average routine activity cost of 350 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (Puckett and Dill 1985). In a 16-h day the fish would consume 5600 mg $O_2 \cdot kg^{-1}$. The cost per charge is 5 mg $O_2 \cdot kg^{-1}$. To double the daily activity cost the fish would need to charge 1120 times (every 51 s). Even including feeding motions this is unlikely. In fact, territorial fish charge only about 12 times per hour (9 foraging plus 3 aggressive), resulting in only 192 charges in 16 h (Puckett 1983).

Furthermore, 14 consecutive bursts increased the routine metabolic rate by only about 30% for the 1st h. The scope for activity is far larger than this so that the fish can be repaying the glycogen debt and still remain very active aerobically. In addition, young coho have a greater aerobic capacity than adult fish (Giles and Randall 1980) and this may help them cope with the large cost of territorial activity.

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Larval Cohort Succession in Herring (*Clupea harengus*) and Capelin (*Mallotus villosus*)

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Lambert, T. C. 1984. Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 41: 1552–1564.

Complexities of herring (*Clupea harengus*) stocks previously alluded to are here explored in detail. Analysis of polymodal larval populations of both herring and capelin (*Mallotus villosus*) from a number of localities reveals the presence of a succession of larval cohorts over time. Separation of these cohorts in time and space is quantified and similarities and differences between species are indicated. The concept of "feeding range" can be applied in explanation of this cohort phenomenon. I suggest that this reproductive strategy serves to limit intraspecific competition and divides a limited food supply more equitably among the total larval population.

La complexité des stocks de hareng (*Clupea harengus*) dont nous avons parlé précédemment est ici explorée en détail. L'analyse des populations larvaires polymodales du hareng et du capelan (*Mallotus villosus*) provenant d'un certain nombre de localités révèle une succession de cohortes larvaires au cours du temps. La séparation de ces cohortes dans le temps et dans l'espace est quantifiée et l'on montre les similarités et les différences entre les espèces. Le principe de la "zone d'alimentation" peut être appliqué pour expliquer ce phénomène des cohortes. Je prétends que cette stratégie reproductive permet de limiter la concurrence et de diviser plus équitablement des approvisionnements alimentaires limités entre les populations larvaires.

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Although recognizing that herring (*Clupea harengus*) spawned at various times during the year, early researchers considered the total northeast Atlantic population a single biological unit. However, around the turn of the century, consistent differences in certain morphological characteristics were discovered. Investigations proceeded into quantitative differences such as vertebral and gill raker counts and qualitative variations in scale rings. Eventually the total herring population was subdivided into groups on the basis of physiological and ecological as well as morphological characteristics (Hodgson 1957; Blaxter 1958; Parrish and Saville 1965). Thus, discrete groups of herring are widely recognized, which, although often mixed on feeding grounds, segregate and move to spawning areas at differing times and places during the year.

These are indications, however, that herring populations are even more complex in structure than hitherto imagined. Probably the first of such evidence was published in a paper on the techniques of analysis of frequency curves, where Buchanan-Wollaston and Hodgson (1929) warned that a great deal of useful information was lost by curve smoothing and combining of data. They contended that "there is strong evidence that the 'roughnesses' in the original data of fish measurements, however small the samples may be, are significant." A portion of their paper concerns the reanalysis of data on juvenile herring captured in the Tamar and Tavy river estuaries in the south of England. It was evident from length–frequency histograms that there were "several broods of young herrings in the catches." The modes in the distribution when plotted over time lie on or near four parallel lines (Fig. 1). "Thus it is more rational to assume that the measurements relate to four broods of young herrings, each having the same growth rate, than to one brood."

Although the polymodal nature of length–freqneucy distributions is obvious in much of the published data on larval herring, it was not specifically addressed until Ware and Henriksens' (1978) study of the structure of the southern Gulf of St. Lawrence herring stocks. They gave evidence that showed the "spring" and "autumn" stocks segregate into two discrete spawning groups, and that each group in turn segregated into two or more runs. Their inferred classification scheme was consistent with the seasonal size distribution of larvae in that larval groups corresponded in time to parental spawning groups.

My objective was to investigate such complexities of population structure involving the production of multiple broods or larval cohorts by examining three independent data sets on larval hering and one of larval capelin (*Mallotus villosus*).

Study Sites and Methods

The data used in this paper are from three locations (Fig. 2); St. Georges Bay, N.S. (this study), St. Marys Bay – Bay of Fundy region (Das 1968), and the Georges Bank – Nantucket Shoals region (Saila and Lough 1981).

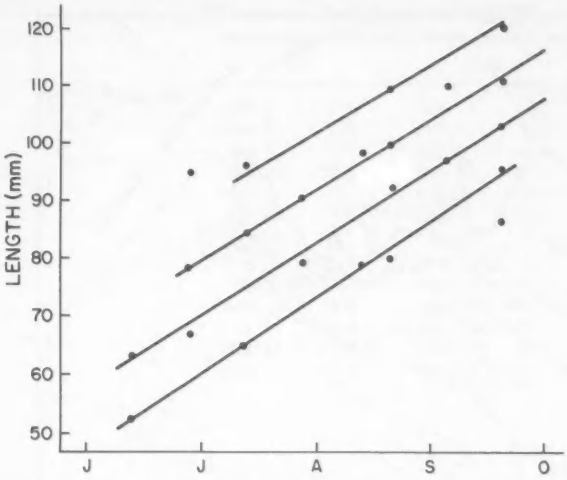

FIG. 1 Juvenile herring cohorts, Plymouth area, U.K. Reproduced from Buchanan-Wollaston and Hodgson (1929).

St. Georges Bay is about 25 km square and 30 m deep. It is fairly representative of the southern Gulf of St. Lawrence but is a discrete body of water insomuch as a clockwise gyre induced by a west to east flow along Northumberland Strait across the mouth of the bay (Petrie and Drinkwater 1978a, 1978b) tends to retain planktonic organisms within the bay. Sampling was conducted during ice-free months (generally May–December) from 1973 to 1976 inclusive at the surface, at 5 and 10 m, and by oblique hauls from the bottom to surface. A neuston sampler (Sameoto and Jarosynski 1969) and 0.5-m ring nets (Lambert et al. 1982a) were used for the horizontal tows, and for the oblique hauls a 6-ft (1.83 m) Isaacs–Kidd midwater trawl (IKMT) was used. The average number of stations (selected randomly) was 12 and each consisted of a 1 nautical mile (1.853 km) tow at 4 knots (7.4 km/h), a bathythermograph cast, and vertical net haul. Mesh size for all nets was 405 μm, except for the vertical net whose mesh was 80 μm. Samples were preserved in 5% buffered formalin and returned to the laboratory where fish larvae were removed and measured.

St. Marys Bay is long and narrow and opens onto the Gulf of Maine but is also connected to the Bay of Fundy by two narrow passages. Its depth gradually decreases from about 35 m at the mouth to 3–5 m at the closed upper end. The bay is exposed to the extreme tidal conditions that are a feature of the Fundy region and receives a portion of the water leaving the Gulf of Maine circulation and entering the Bay of Fundy along the southwestern Nova Scotia shore. During 1961–63, 23 stations within St. Marys Bay were occupied monthly, and 5 stations on a transect between Grand Manan Island and Briar Island at the mouth of St. Marys Bay were visited at irregular intervals. Samples were collected with 1-m-diameter No. 0 nylon open and closing nets and a 6-ft (1.83 m) IKMT. Stepped tows were made with these nets at 20 and 10 m and at the surface. Temperature and salinity were also recorded. Samples were preserved in 5% formalin and larvae were removed later and measured.

Georges Bank is a well-known fishing ground and is the largest of the three areas I considered; on average, 90 000 km^2 were sampled each survey. These data were collected in 1973 and 1974 as part of an extensive larval herring survey initiated in 1971 under the auspices of the Northwest Atlantic Fisheries Organization (formerly ICNAF). At each station, oblique tows were made to within 5 m of the bottom to a maximum depth of 100 m. The 61-cm bongo net (505- and 333-μm mesh nets) was deployed at 3.5 knots (6.5 km/h). Conductivity–temperature–depth casts were made at selected stations to within 10 m of the bottom. Additional details on sampling and locality for St. Georges Bay can be found in Lambert et al. (1982a) and for the Georges Bank – Nantucket Shoals region in Lough et al. (1979).

In all studies, larvae were grouped in 1-mm size categories. Length–frequency histograms were constructed from raw data, and in most cases strong polymodality was evident. Populations were resolved into components using the method of Harding (1949), later modified by Cassie (1954). In some cases where groups of larvae were distinct from the main body of the population and when the population was obviously unimodal, modes were selected by eye.

Analytical Technique

The problem of analyzing polymodal frequency distributions in biology has been discussed at great length and many methods proposed since perhaps the earliest treatment of the subject by Buchanan-Wollaston and Hodgson (1929). A cross-section of methods can be found in Tanaka (1962), Taylor (1965), Bhattacharya (1967), Harris (1968), and MacDonald and Pitcher (1979). All methods have advantages and disadvantages; I have chosen the graphical method of Harding (1949) modified by Cassie (1954) because it is relatively simple and works well for most of the data presented here. Its most difficult application occurs when a small component population lies adjacent to a much larger component and becomes almost hidden in the flank of the latter. In general, all components are closely spaced but for the most part are about equal in size, so readily identifiable.

Other methods of analysis were tried in a few difficult situations with little improvement. Clearly what is needed is reorganization of the data into smaller size catagories. This would confer two advantages: space between modes would increase and reduce the masking effect of a large mode on a small one and the number of degrees of freedom would increase, thus giving a better statistical fit (three degrees of freedom are lost for each component population extracted from the complex). Unfortunately, measurements of larval fish grouped in size-classes smaller than 1 mm are rare; indeed, the converse is quite common, such as intervals of 5 and 10 mm.

The statistical fits of the extracted solutions of the polymodal populations to original data were poor. In χ^2 tests most probabilities fell between the 10 and 5% levels. Given only one polymodal population, the validity of the component breakdown would be doubtful. However, when presented with a succession of such populations, we are able to apply what Buchanan-Wollaston and Hodgson (1929) termed the "test of consistency." First, if the modes are significant and represent a real subgroup, there should be a repeatable pattern and progression in position of modes from month to month. Second, parts of a sample should show the same modes as the whole. Both of these constraints are satisfied. A good example to illustrate the first condition can be found in the St. Marys Bay data for November 2 and December 1, 1960. The spacing and relative proportions of three modes are identical: 15, 17, and 19 mm in the former and 21, 23, and 25 mm in the latter.

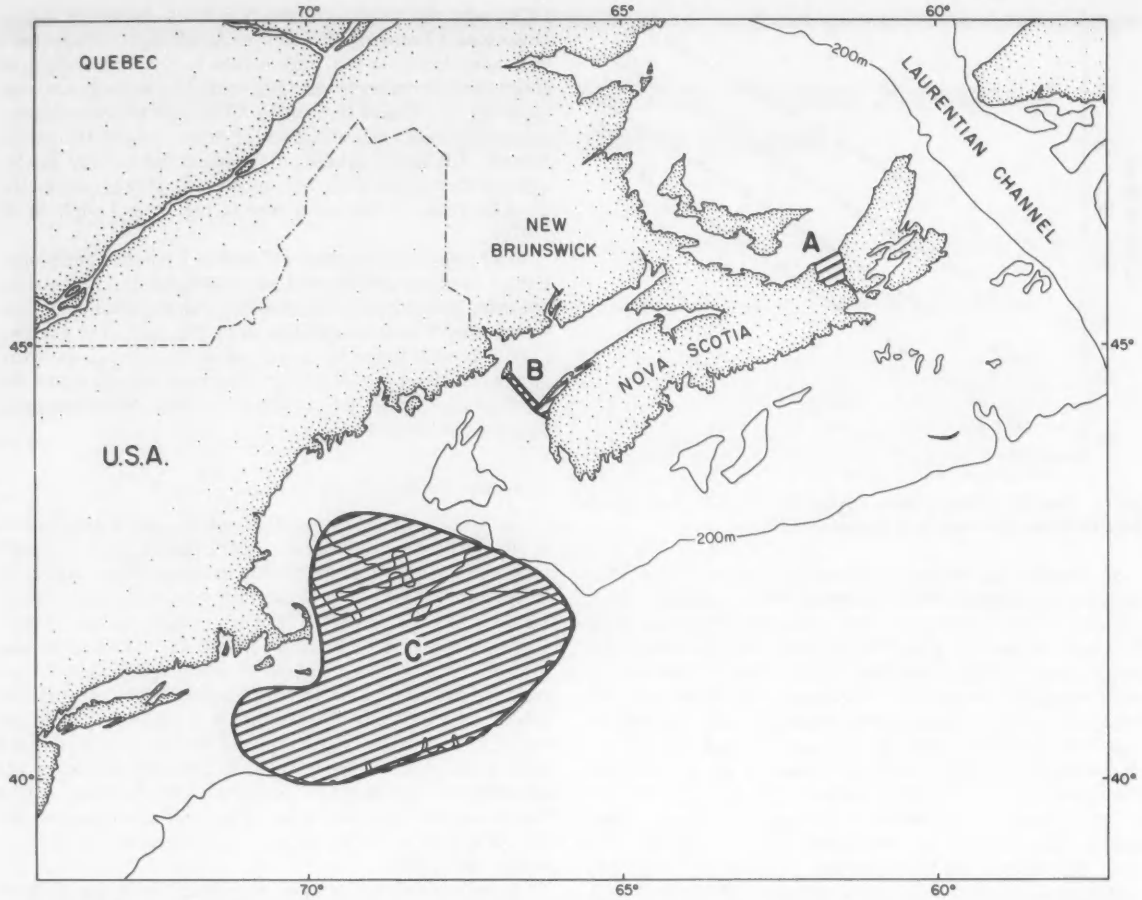

FIG. 2. Location of sampling areas. A, St. Georges Bay; B, St. Marys Bay – Bay of Fundy; C, Georges Bank – Nantucket Shoals.

To test the second condition, in St. Georges Bay, I analyzed data from the IKMT separately from that gathered by horizontal tows. Apart from the addition of some modes in large-size larvae (IKMT is more efficient at capturing bigger larvae) the solutions were identical.

In some cases alternative growth lines can be plotted between the monthly component means; however, most of these give completely unrealistic growth rates.

Although the interpretation I present here seems quite complex, the consistency, both within and between data sets, plus the close agreement of derived growth rates with those of other investigations demonstrate the effectiveness of the methods I have used; in many ways the results of the analysis justify its use.

Results

Herring Larvae, St. Georges Bay

During the middle 1970's both spring and autumn stocks in the southern Gulf of St. Lawrence declined to all time lows (Winters 1976; Lambert et al. 1982b). This was reflected in larval abundance in St. Georges Bay (Table 1). Larvae from both spring and autumn spawning within the bay were represented as well as some immigrant larvae that had drifted east along Northumberland Strait from summer spawning in the Pictou area some 80 km west of St. Georges Bay. Lambert et al. (1982b) estimated that the bay received about 0.2% of the larvae produced near Pictou. The relative densities of the three larval groups are shown in Fig. 3.

Warming is extremely rapid in the bay in early summer and although there may be as much as a 10°C difference in temperature between surface and bottom initially, the water column generally becomes isothermal by the end of August. Surface temperatures are presented in Table 2.

The polymodal nature of the populations and monthly progression of modes over time are illustrated in Fig. 4. Each population was resolved into components and the mean size of each component plotted for each sampling date (Fig. 5). Lines were fitted over time to these points, revealing a number of fairly equally spaced larval cohorts. Even though larval cohorts were clearly evident in 1975 and 1976 the paucity of larvae precluded any formal analysis of the data other than plotting a few modes by eye. The mean growth of the larvae in 1973 and 1974 was 0.21 and 0.19 mm/d, respectively.

Herring Larvae, St. Marys Bay – Bay of Fundy

The larvae collected in this study originated mainly from

TABLE 1. Mean density (no./1000 m^3) of larval herring in St. Georges Bay, 1973–76.

1973		1974		1975		1976	
June 1	9.4	June 14	2.3	June 12	0.8	May 12	8.6
June 8	5.9	June 25	0.4	June 18	0	May 18	7.3
June 21	2.7	July 4	0.3	June 24	0.1	May 27	1.5
June 29	11.4	July 11	1.7	July 3	0	June 1	14.6
July 10	0	July 29	3.4	July 8	0	June 8	2.2
July 20	0	Aug. 8	0.1	July 15	0	June 15	5.0
July 26	0	Aug. 13	1.6	July 23	0	June 22	0
Aug. 3	7.1	Aug. 20	0.3	July 31	0	June 29	0
Aug. 8	0	Sept. 11	0.9	Aug. 6	0.6	July 6	2.3
Aug. 18	8.6	Sept. 25	11.1	Aug. 12	2.0	July 15	1.0
Oct. 3	1.2	Oct. 7	22.4	Aug. 19	4.0	July 20	0.7
Oct. 25	19.8	Nov. 20	86.1	Aug. 26	2.1	July 27	0
Dec. 9	55.1	Dec. 12	116.1	Sept. 23	1.4	Aug. 3	0
						Aug. 18	3.4
						Aug. 23	2.2
						Sept. 8	0.4
						Sept. 21	3.2
						Oct. 6	0
						Nov. 16	25.4

TABLE 2. Mean surface temperature (°C) in St. Georges Bay, 1973–76.

1973		1974		1975		1976	
June 1	6.5	June 14	10.3	June 18	9.6	May 12	5.5
June 8	8.4	June 25	12.4	June 24	12.5	May 18	5.7
June 21	11.5	July 4	14.1	July 3	15.4	May 27	6.5
June 29	15.2	July 11	14.7	July 8	19.4	June 1	8.3
July 10	16.4	July 25	16.7	July 15	19.6	June 8	12.3
July 20	17.8	July 29	15.9	July 23	20.1	June 15	10.5
July 26	18.6	Aug. 8	18.8	July 31	18.7	June 22	14.0
Aug. 3	19.5	Aug. 13	19.4	Aug. 6	19.5	June 29	14.5
Aug. 18	19.2	Aug. 20	20.1	Aug. 12	19.5	July 6	15.5
Oct. 3	13.5	Sept. 11	17.9	Aug. 19	19.4	July 15	16.1
Oct. 25	12.0	Sept. 25	16.5	Aug. 26	18.0	July 20	17.2
Dec. 9	4.7	Oct. 7	14.0	Sept. 23	16.7	July 27	17.5
		Nov. 20	7.1			Aug. 3	17.9
		Dec. 12	4.6			Aug. 23	19.3
						Sept. 8	17.1
						Oct. 6	16.2
						Nov. 16	6.6

autumn spawning off southwest Nova Scotia and to a much lesser extent from summer spawning off Grand Manan in the mouth of the Bay of Fundy (Das 1978) and spring spawning at the head of St. Marys Bay (Tibbo et al. 1958; Boyar 1968). Das (1968) did not present larval densities as such; the following are my estimates made from his tables of larval abundance according to net. The volumes filtered by the sampling gear were estimated to be 730 m^3 for the 1-m ring net and 1000 m^3 for the IKMT (see Table 3).

The water in this area is well mixed and temperature varies little from surface to bottom (the maximum difference during the study period was 3°C). Surface values occurring a few kilometres offshore appear in Table 4.

Histograms and progression of cohort mean size over time are given in Fig. 6 and 7. In all years, growth slowed during the winter and increased in early summer. Mean rates derived from Fig. 7 are shown in Table 5.

Herring Larvae, Georges Bank – Nantucket Shoals

These larvae originate from autumn spawning on Georges Bank. The abundance of larvae during an 8-yr period beginning in 1971 was highest in 1973, the year used in this analysis; densities were reported to be 11–1000/m^2. Water temperature was higher than in southwest Nova Scotia, but similarly, there was little difference between surface and bottom. Mean temperatures at 20 m during the sampling period 1973–74 were taken from Bolz and Lough (1981) and are as follows: 15.8 (October 9), 13.3 (November 2), 10.6 (December 13), 6.2°C (February 14).

Length–frequency histograms and cohort mean sizes over time appear in Fig. 8 and 9. Cruises were generally about 2 wk long; thus, the midpoint of each was chosen as the sample date for Fig. 9.

Mean growth rates derived from Fig. 8 were 0.22 (Septem-

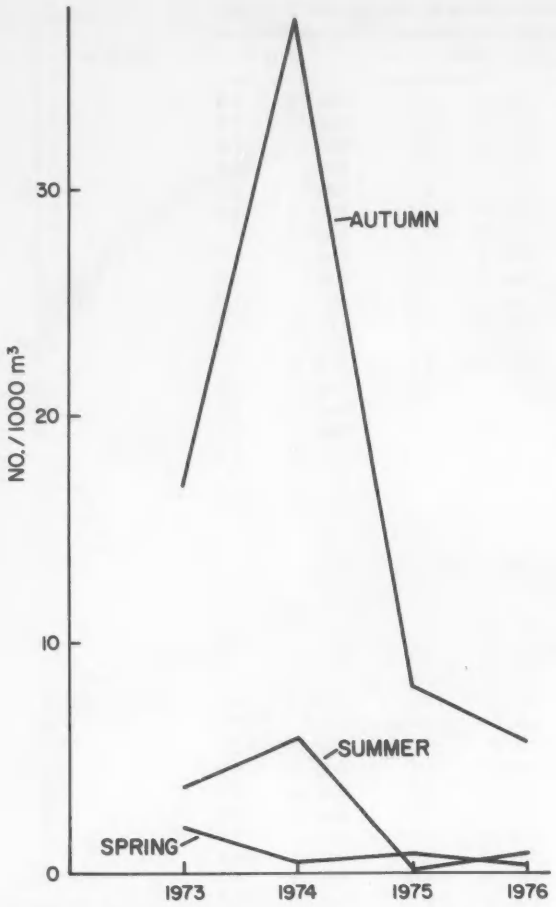

FIG. 3. Mean annual densities of larval herring groups in St. Georges Bay. Derived from neuston and 0.5-m ring net samples at 0, 5, and 10 m, and samples from an obliquely hauled Isaacs–Kidd midwater trawl (see Lambert et al. 1982a).

ber–October), 0.08 (November), and 0.06 mm/d (December–February).

Capelin Larvae

There was an unusual occurrence of capelin in St. Georges Bay during the mid-1970's. This was probably because exceptionally large year-classes at that time (Carscadden 1983) caused an expansion of the distribution of the stock farther south than normal. A few larvae were detected in 1973, and thereafter numbers increased in successive years (Table 6). The numbers of adult fish peaked in 1977; a few were detected in 1978 but none thereafter (Lambert et al. 1982b).

Capelin larval data were analyzed in a manner identical to those for herring. Results are shown in Fig. 10 and 11. Over the time that larval capelin were sampled their apparent seasonal growth rates were constant; the mean rates for 1974–76 were 0.39, 0.29, and 0.35 mm/d, respectively.

Origin of Herring Cohorts

The observed succession of larval cohorts could be a result of

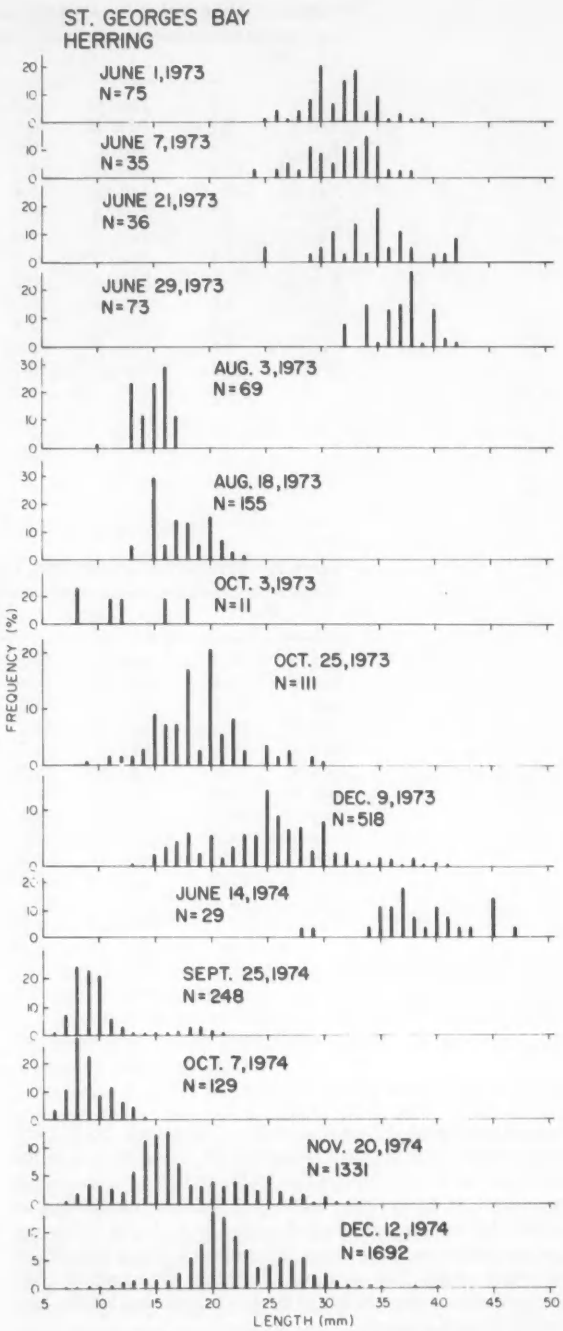

FIG. 4. Length–frequency histograms of larval herring, St. Georges Bay, 1973 and 1974.

adult spawning behavior or induced by the environment in some way. Ware and Henriksen (1978) have shown that runs (peaks in landings) of herring coincided with the origin of larval cohorts. This evidence, although convincing, is, nevertheless, circumstantial. To strengthen the case, I examined the spawning

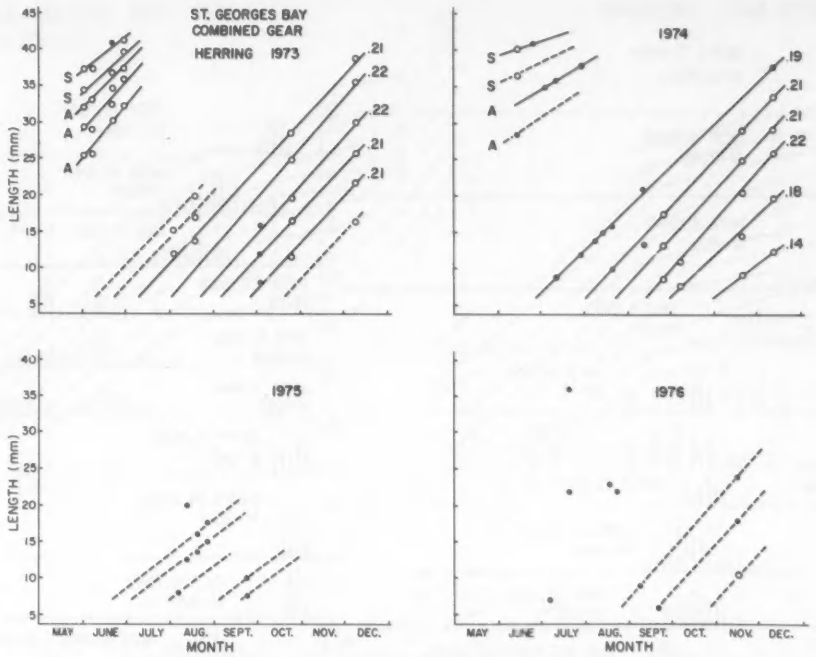

FIG. 5. Larval herring cohorts, St. Georges Bay, 1973–76. Points are mean lengths of component populations. ○, derived from analysis (Cassie 1954); ●, plotted by eye. Numbers to the right are growth rates (mm/d) of larval cohorts; S and A are summer- and autumn-spawned cohorts from previous year.

TABLE 3. Larval herring density (no./1000 m^3) in St. Marys Bay, 1960–63. IKMT = Isaacs–Kidd midwater trawl.

1960–61	1-m net	IKMT	1961–62	1-m net	IKMT	1962–63	1-m net	IKMT
Sept.	1890		July	—	4	Sept.	85	1
Oct.	90		Aug.	2	0.5	Oct.	42	2
Nov.	9		Sept.	70	35	Nov.	1	13
Dec.	4		Oct.	11	7	Dec.	0.5	8
Jan.	0.3		Nov.	3	3	Feb.	0.2	—
Feb.	0.1	5	Dec.	2	2	Apr.	0	3
Mar.	0.2	7	Feb.	—	1	May	7	49
Apr.	1	42	Apr.	—	57	June	0	0.8
May	—	150	May	—	49			
June	—	7	June	—	0.2			

TABLE 4. Monthly mean surface temperature (°C) at Lurcher lightship, approximately 28 km southwest of St. Marys Bay.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1960	4.2	2.6	2.2	3.1	5.6	8.2	10.1	10.4	—	—	—	6.8
1961	4.3	1.7	1.3	1.8	3.7	5.8	8.6	9.4	10.0	10.5	—	7.1
1962	4.9	2.3	1.5	2.9	4.9	7.3	8.6	10.0	—	—	8.0	7.2
1963	4.6	4.3	3.1	4.2	4.8	7.6	9.6	12.5	—	—	8.3	6.4

condition of spring herring over a 6-wk period. Three peaks of ripe and running fish followed closely by three peaks of spent fish (Fig. 12) indicated three discrete egg depositions. The three inferred spawning peaks coincided with peaks in landings, and the spacing between these peaks was consistent with the spacing between larval cohorts.

An examination of data presented by Jean (1956) yields similar results. A number of spawning waves are evident in the spring of three years (Fig. 13). This phenomenon cannot be discerned during the autumn, for although there appears to be some rhythmicity in the proportion of ripe and running fish, there is none in spent fish due to the almost complete absence of

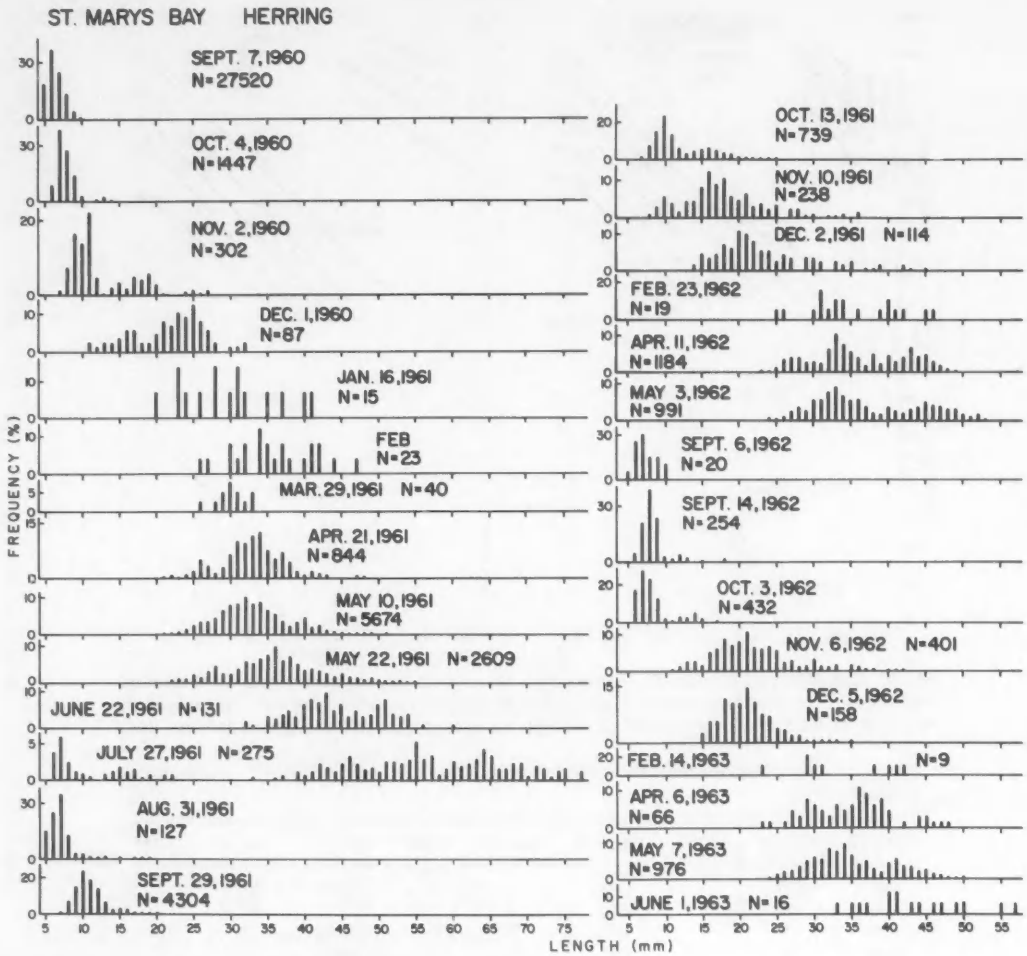

FIG. 6. Length–frequency histograms of larval herring in St. Marys Bay, 1960–63.

TABLE 5. Mean seasonal growth rate (mm/d) of larval herring in St. Marys Bay.

	Sept.–Nov.	Dec.–Mar.	Apr.–May	May–June	July
1960–61	0.21	0.10	0.02	0.13	0.30
1961–62	0.18	0.05	0.09	—	—
1962–63	0.19	0.09	0.08	—	—

the latter in the samples. Jean (1956) suggested that, in contrast with the behavior of spring spawners, fall spawners leave the spawning grounds and move to deeper water as soon as they have spawned.

During Jean's (1956) 3-yr study the number of spring spawning waves decreased from four to two. The times between waves were 17, 17, and 18 d in 1952, 16 and 35 d in 1953, and 34 d in 1954. Since the larger spacing is double the smaller, it seems that runs originally present failed to materialize in subsequent years. Interestingly, landings of adult herring fell during this period; this was also reflected in larval abundance (mean numbers per tow, 1952–54, were 230.4, 49.6, and 13.2, respectively).

Dempsey and Bamber (1983) examined a herring spawning bed in the Blackwater Estuary in England. Grab samples were taken over a 2-wk period followed by plankton samples when hatching began. Their data clearly show three discrete spawnings spaced 6 and 21 d apart.

From the data I have presented here and that of Ware and Henriksen (1978) I suggest that herring spawning groups (i.e. spring, summer, and autumn spawners) segregate into three or four runs or spawning waves. Thus, in St. Georges Bay, for

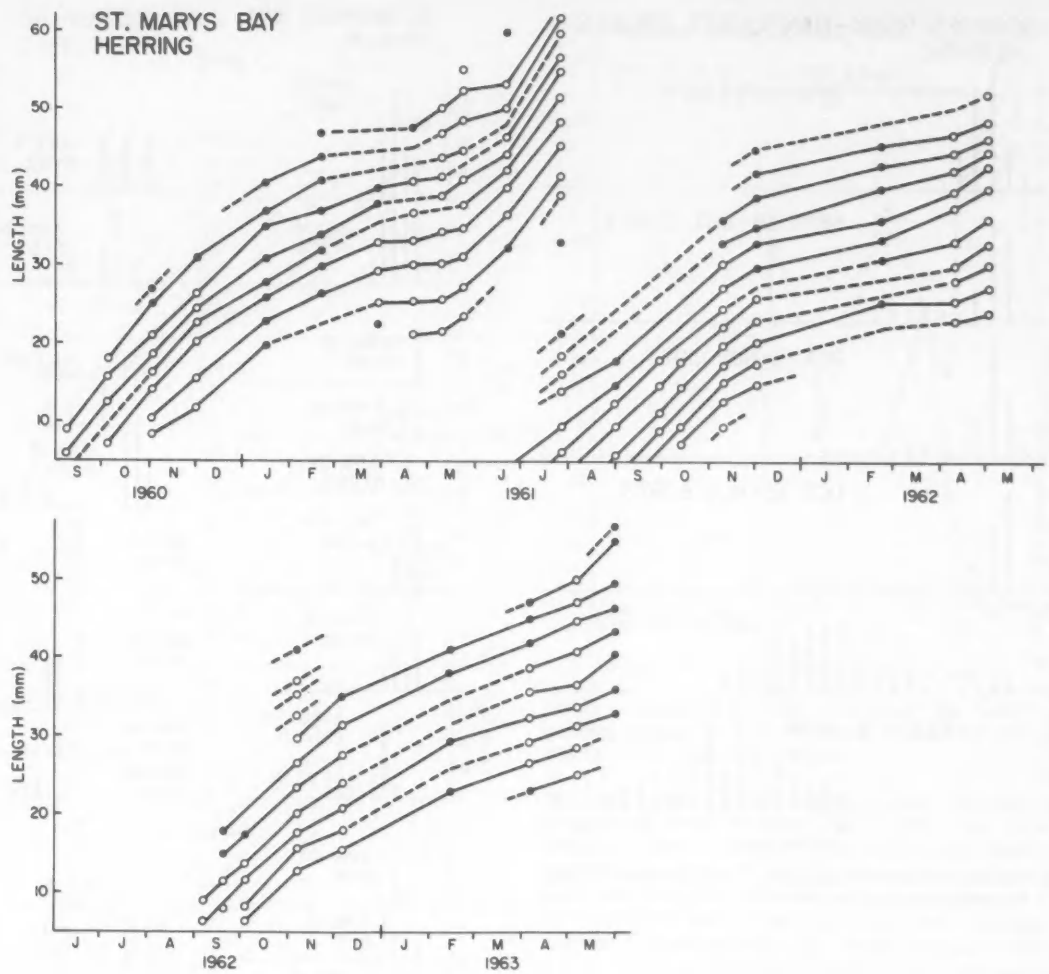

FIG. 7. Larval herring cohorts, St. Marys Bay, 1960–63. Symbols as in Fig. 5.

TABLE 6. Density of capelin larvae (no./1000 m^3) in St. Georges Bay, 1973–76.

1973		1974		1975		1976	
June 1	0	June 14	0.5	June 12	30.7	May 12	0.3
June 8	0	June 25	28.6	June 18	3.1	May 18	2.0
June 21	1.6	July 4	22.3	June 24	66.5	May 27	1.3
June 29	3.5	July 11	14.8	July 3	21.9	June 1	4.7
July 10	0	July 29	12.3	July 8	7.3	June 8	1.3
July 20	0	Aug. 8	0.4	July 15	3.9	June 15	72.4
July 26	0	Aug. 13	0	July 23	2.8	June 22	837.7
Aug. 3	0	Aug. 20	0.1	July 31	8.2	June 29	523.0
Aug. 8	0	Sept. 11	0.2	Aug. 6	12.6	July 6	137.8
Aug. 18	0.3			Aug. 12	14.2	July 15	83.3
				Aug. 19	12.8	July 20	57.2
				Aug. 26	7.2	July 27	5.4
				Sept. 23	0.6	Aug. 3	0.2
						Aug. 18	0
						Aug. 23	0
						Sept. 8	0.2

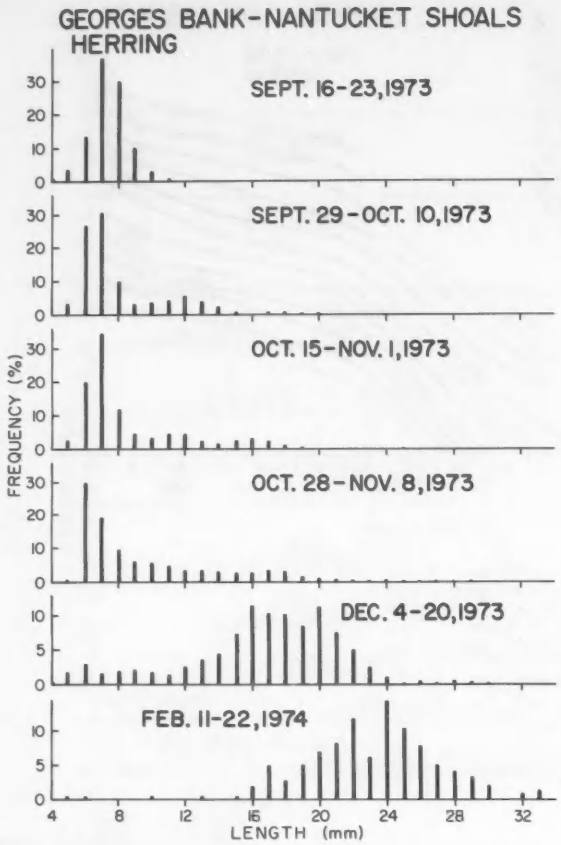

FIG. 8. Length–frequency histograms of larval herring in Georges Bank – Nantucket Shoals region, 1973–74.

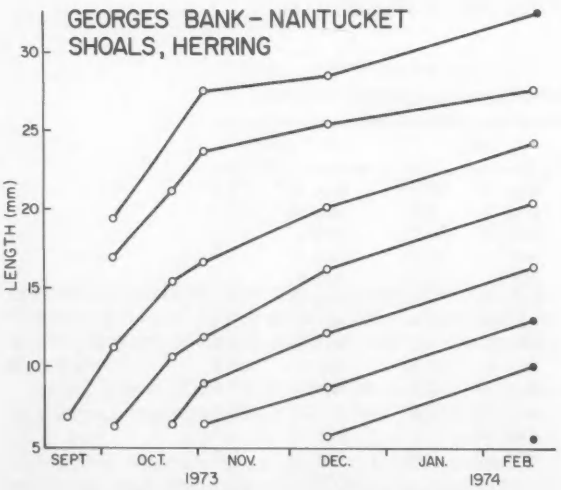

FIG. 9. Larval herring cohorts, Georges Bank – Nantucket Shoals, 1973–74. Symbols as in Fig. 5.

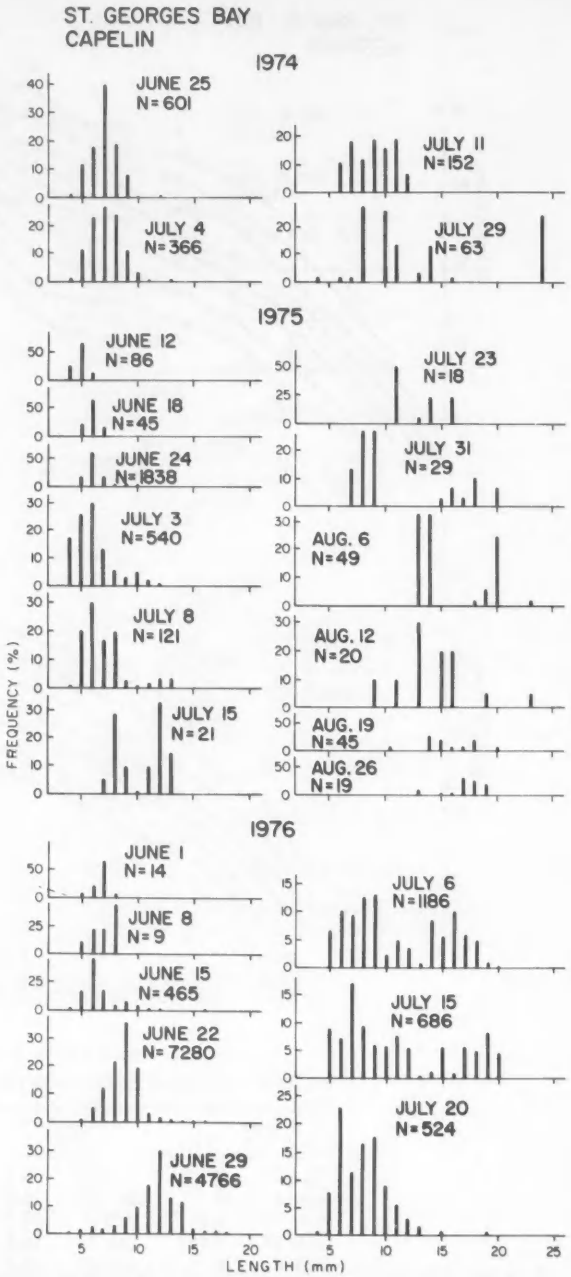

FIG. 10. Length–frequency histograms of larval capelin in St. Georges Bay, 1974–76.

example, where two groups and part of the third (summer) are represented, up to eight larval cohorts can be detected.

Origin of Capelin Cohorts

The occurrence of capelin in St. Georges Bay was highly unusual and so there was no established or accessible market for the fish. Hence, they were but a nuisance to fishermen and were

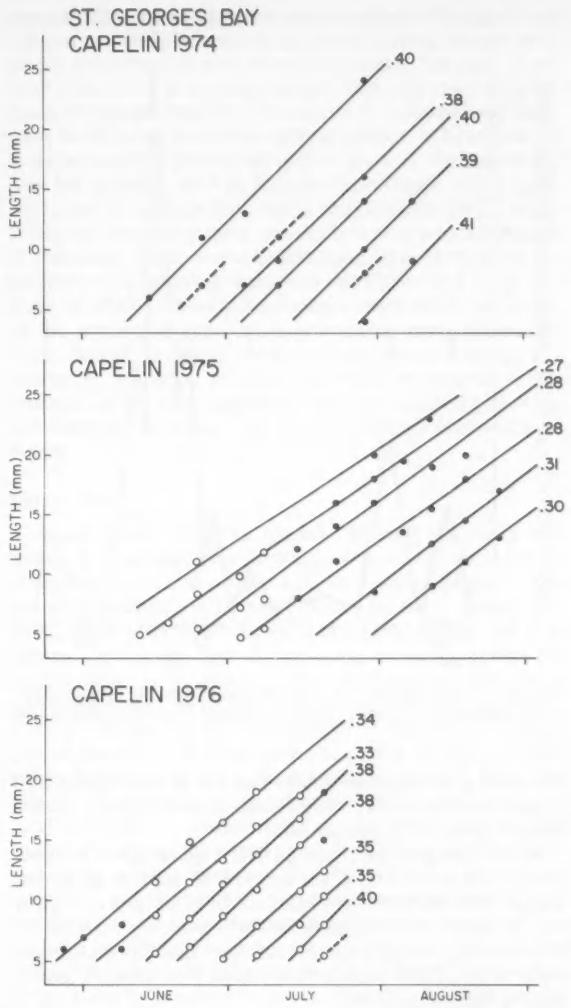

FIG. 11. Larval capelin cohorts, St. Georges Bay, 1974–76. Symbols as in Fig. 5.

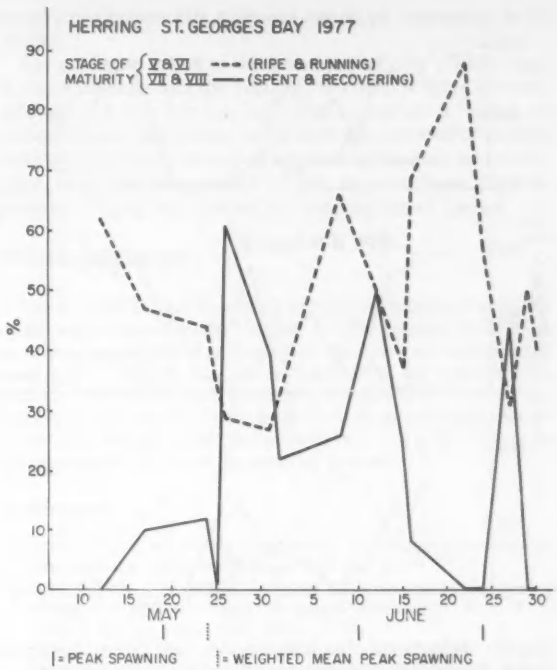

FIG. 12. Proportions of "ripe and running" and "spent" spring spawning herring collected by trap net, St. Georges Bay, 1977. Mean peak spawning weighted by landings.

discarded from trap nets with no record of abundance kept. Furthermore, since most capelin die after spawning, there did not seem to be much point in assessing maturity index as was done for herring. Nevertheless, it was clear, especially when capelin were abundant in 1977, that the fish arrived in two waves about 2 wk apart (Lambert et al. 1982a). Frank and Leggett (1981) also reported two waves of spawning capelin entering Bryants Cove, Nfld., in both 1978 and 1979, spaced 15 and 12 d apart, respectively. In 1975 and 1976 in St. Georges Bay, six and seven cohorts, respectively, could be detected in the plankton. In Newfoundland, Frank and Leggett (1981) monitored the release of capelin larvae from beach gravel by towing nets in the intertidal zone. Their fig. 4 is reproduced here, slightly modified (Fig. 14), and depicts abundance of larvae immediately above the gravel spawning bed. Any peak greater than an arbitrarily selected background or "noise" level of 500/m^3 is considered a larval release and is marked by an arrow. The large complex peak spanning mid-July is considered one peak, since it would appear as such when the larvae dispersed farther from shore. Thus, five peaks, one being dominant, can be identified and would appear later in the plankton as successive cohorts. This pattern of release is identical to that observed in St. Georges Bay where one cohort was dominant (>80% of total larvae) each year. The mean (SD) spacing of capelin cohorts in St. Georges Bay was 11.6 d (3.5) and 8.8 d (2.5) in 1975 and 1976, respectively, comparing well with 9.0 d (SD 2.4) observed in Bryants Cove.

The spacing between capelin cohorts is less than the time between spawning waves, and furthermore, whereas no more than two spawning waves were observed, at least five larval cohorts were present on all occasions. Frank and Leggett (1981, 1982) have clearly shown that the release of capelin larvae from beach gravels is related to the occurrence of onshore winds, a finding that was corroborated by Lambert et al. (1982a). Thus, the proximal cause of successive larval cohorts in capelin is abiotic, in contrast with herring.

Cohort Spacing

The spacing of cohorts has some interesting features. At any particular moment the cohorts are distinct or separated on the basis of size; this can be thought of as separation in space. Also, there is a separation in time between the release or origin of each cohort.

The space separation is markedly constant; the range including both herring and capelin is narrow. The lowest mean seasonal value was 2.79 mm and the highest was 4.36 mm. Nevertheless, there is a significant difference between species: 2.81 mm (SD 0.19) for capelin and 3.39 mm (SD 0.71) for herring (significant at 1% level, t = 3.01, 59 df). Jean (1956)

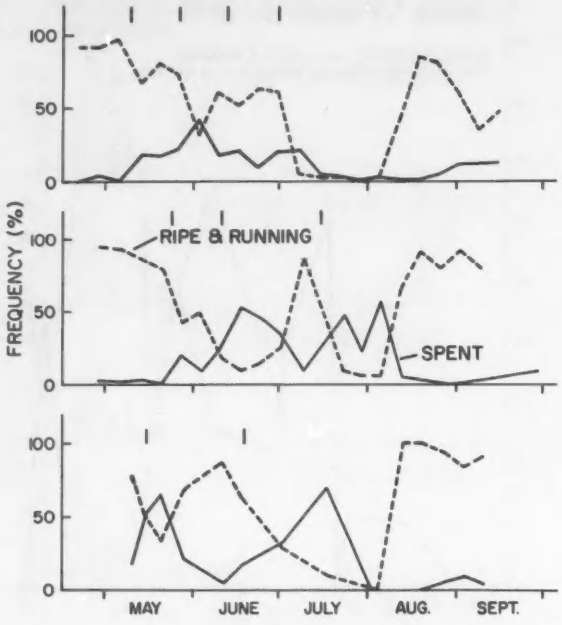

FIG. 13. Proportions of "ripe and running" and "spent" herring at Grande Riviere, Bay of Chaleur, 1952–54, top to bottom. Vertical bars indicate spawning events as in Fig. 12. Redrawn from Jean (1956).

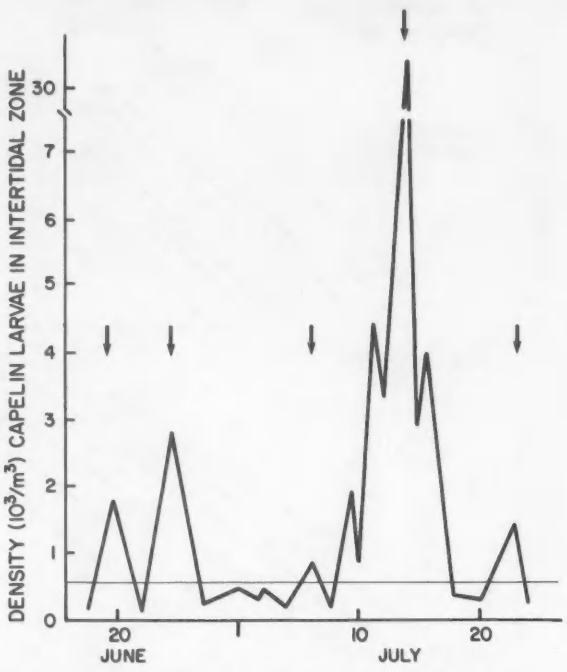

FIG. 14. Larval capelin releases from beach gravel, Bryants Cove, 1978. Arrows mark cohorts. Slightly modified from Frank and Leggett (1981).

analyzed herring populations in the Bay of Chaleur in the west of the Gulf of St. Lawrence over a 3-yr period. The populations were polymodal as shown by modal lengths marked on the overall range in size for each sample period. Although the data were not detailed enough to allow the plotting of cohort lines over time, the distance between modes could be measured. Thus, the mean spacings for 1952–54 were 3.39, 3.24, and 3.50 mm, respectively, in close agreement with the values I measured.

The spacing over time is more variable; seasonal means including both herring and capelin range from 7.9 to 25.2 d. The origin of cohorts was determined by extrapolating backwards to size at hatching where practicable (i.e. in all cases where growth rate was constant, but not when growth lines were curvilinear or when the smallest size mode was greater than 10 mm). Again there was a significant difference between capelin and herring: 10.09 d (SD 3.18) and 17.50 d (SD 6.53), respectively (significant at 1% level, $t = 3.55$, 33 df).

Discussion

Role of Cohorts

The cohort system including interspecific differences in spacing can be conveniently explained using an hypothesis of Jones and Hall (1974) involving the concept of "feeding range." Briefly, they suggested that cohorts of fish larvae tend to grow up with cohorts of copepods and that the diet of fish larvae is restricted to a certain size range of copepods (the upper limit of which would be determined physically by mouth size and the lower limit physiologically by foraging costs). This size range of available food (the feeding range) represents the production of copepods over a period of time and can be expressed in days. The feeding range thus represents the interval required between two cohorts to minimize competition due to overlapping food ranges. In other words, it is the time required to keep adjacent cohorts nutritionally independent.

In St. Georges Bay, herring and capelin hatch at about 7 and 5 mm, respectively. The mean mouth gape (as defined by Shirota 1970) for herring <9 mm was 0.34 mm and for capelin <7 mm was 0.25 mm (significant at 1% level, $t = 5.15$, 19 df). Thus, herring, having larger mouths, are capable of exploiting a wider range of food particles and require proportionally greater spacing between cohorts.

The interval between consecutive releases of larvae does not appear to be related to egg development rate for either capelin or herring. In nearly all cases this interval is greater than the development time predicted at ambient temperatures. Rather, it appears the goal of the cohort system is to maintain the size separation between the cohorts, since that value varies little. This spacing is in effect a product of larval growth rate and length of interval between cohort releases. Thus, the faster the growth rate, the closer the cohort releases can be.

The production of a succession of larval cohorts could confer other advantages on these demersal spawners. Fishes are most vulnerable when they are larvae, so those species having protracted larval stages, herring in particular, are at a disadvantage. Thus, the cohort system can be thought of as a "hedged bet" strategy that spreads the spawning effort over time to take advantage of a variable environment, an idea further developed by Lambert and Ware (1984).

Origin of Cohorts

Apparently, larval cohort succession, with the presumed described benefits, has been arrived at differently by the two

species. The causes appear to be completely biological and linked to spawning behavior in the case of herring, but primarily abiotic and related to wind events for capelin, although, in the latter case, there is a biological subcomponent involved. The actual release of larvae from beach gravel is caused by onshore wind but the range over time, or total number, of larval cohorts is made possible by waves of spawning adults. Two spawning runs are reported, both in Bryants Cove (Frank and Leggett 1981) and St. Georges Bay. Saetre and Gjøsaeter (1975) report of Barents Sea capelin there are usually several runs at a number of localities. Wave action redistributes eggs throughout the intertidal zone, moving them between higher and lower tide levels at which diffential development rates occur, depending on the amount of exposure to warmer air temperatures (K. Frank, Marine Ecology Laboratory, pers. comm.). Hence, any biological synchrony in hatching would be smoothed out. Instead, a range of developmental stages would result allowing accumulation between, and release during, onshore wind events.

Larval Growth

Where cohorts could be followed through the winter into spring it is obvious that larval growth slows considerably. Nevertheless, it is also clear that often during periods when larvae experience a wide range of temperatures, growth rate often varies little. This occurs in St. Georges Bay for both herring and capelin and off the south coast of England for herring (Fig. 1, 4, and 11). Kerr and Dickie (1985) have investigated this peculiarity using a bioenergetic approach. They concluded that larvae born later in the season realize a greater proportion of their metabolic potential, despite falling temperature, due to a greater availability of food. Thus, the effect of temperature on growth is overridden, or damped, by nutrient limitations. However, when temperatures are low and food scarce during the winter, growth decreases as expected.

Another striking feature of the larval cohort system is that even when growth rates vary they do so in concert, thus keeping the cohorts equidistant. Because of the amazing regularity, it is probable that the original spacing of cohorts (as a result of wind events for capelin and spawning waves for herring) is modified or fine tuned by subsequent factors. Such factors could involve temperature and egg development rate, especially for herring, and perhaps intraspecific competition for both capelin and herring. Consider two closely spaced cohorts: it is entirely possible those individuals in the region where the two distributions overlap fare less well than those in the outlying regions; differential mortality could occur and in effect the modes of the distributions would move apart.

Whatever the regulating mechanism, whether it involves intraspecific competition or some kind of environmental constraint, it is obviously powerful, for it operates on a number of larval fish stocks that experience a wide range of environmental conditions.

Generality of Cohorts

I have presented evidence of a succession of larval cohorts in herring stocks on both sides of the Atlantic. In addition, larval herring cohorts occur in the Pacific at approximately 2-wk intervals in the plankton of southern British Columbian waters (D. M. Ware, Pacific Biological Station, Nanaimo, B.C., pers. comm.). A similar presence in capelin beyond the western North Atlantic remains to be demonstrated, but there seems to be no good reason that it should not be as widespread as in herring.

The pervasive nature of this phenomenon over a wide range of environments, and the fact that it seems to have evolved separately in two species, leads one to suspect a feature of possibly major importance in the early life survival of herring and capelin; perhaps even, not to be confined to these two alone, since even a brief perusal of the literature will disclose polymodal larval populations in a wide variety of species.

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Reproductive Strategies of Demersal and Pelagic Spawning Fish

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Lambert, T. C., and D. M. Ware. Reproductive strategies of demersal and pelagic spawning fish. Can. J. Fish. Aquat. Sci. 41: 1565–1569.

The spawning tactic of herring (*Clupea harengus*) wherein batches of eggs are deposited leading to a succession of larval cohorts is elaborated. We found the time interval between cohorts to be a function of larval growth rate as well as larval mouth size. In view of the relationship with growth rate we suggest a continuum of reproductive strategies with "bet hedging" by herring at one extreme and an "all at once" egg release by Atlantic mackerel (*Scomber scombrus*) at the other.

La tactique de frai du hareng (*Clupea harengus*) est de déposer des groupes d'œufs, ce qui conduit à une succession de cohortes larvaires. Nous avons constaté que l'intervalle de temps entre les cohortes était fonction du taux de croissance des larves ainsi que de la taille de la bouche de ces larves. Étant donné la relation avec le taux de croissance, nous estimons qu'il existe une série continue de stratégies de reproduction allant de l'étalement de la ponte à une extrémité, à la ponte unique du maquereau bleu (*Scomber scombrus*) à l'autre extrémité.

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Fish spawn at various times during the year and newly hatched larvae experience a wide range of environmental conditions. Atlantic mackerel (*Scomber scombrus*) larvae born in the summer generally experience warm temperatures and abundant food whereas autumn-spawned herring (*Clupea harengus*) larvae encounter the opposite. Consequently, mackerel have a much higher growth rate.

A mechanism by which fish can enhance the survival of offspring released in adverse conditions was presented by Lambert (1984) who demonstrated the existence of a succession of larval cohorts in two demersal spawners, herring and capelin (*Mallotus villosus*). He suggested that this was a "bet hedging" strategy that would spread spawning effort over time to take advantage of (or, from the opposite point of view, counteract) environmental variability.

In this paper we expand on these ideas and compare the spawning tactics of the aforementioned demersal spawners to those of two pelagic spawners, mackerel and white hake (*Urophycis tenuis*).

Study Site and Sampling Methods

The sampling site, St. Georges Bay, N.S., is approximately 25 km square and is situated at the southeast of the southern Gulf of St. Lawrence. It is admirably suited to ichthyoplankton studies, being large enough to have diversity of habitat and be reasonably representative of the southern Gulf, yet small enough to allow fine-scale sampling in time and space. Furthermore, a clockwise gyre (Petrie and Drinkwater 1978a, 1978b) retains zooplankton within the bay, allowing accurate estimates of growth and mortality.

Stations were randomly selected for each cruise during exploratory sampling in 1973; thereafter, a grid was established and for each of the next 3 yr fixed stations were randomly selected. As an example, those for 1974 are shown in Fig. 1. A box-type neuston sampler and 0.5-m-diameter ring nets were towed at the surface and 5 and 10 m, and a 6-ft (1.83 m) Isaacs–Kidd midwater trawl (IKMT) was towed obliquely from bottom to surface. All tows were simultaneous over a distance of 1 nautical mile (1.853 km) at a speed of 4 knots (7.4 km/h). At the end of each station the boat was stopped and a vertical haul with a 0.5-m ring net and a bathythermograph cast were made. The mesh size of all nets (including cod-end of the IKMT) was 405 μm, except for the vertical haul net whose mesh was 80 μm. Samples were preserved in 5% buffered formalin and returned to the laboratory for analysis. Further details of site and sampling protocol can be found in Lambert et al. (1982).

Results and Discussion

Lambert (1984) described a succession of larval cohorts in herring and capelin. He applied an hypothesis of Jones and Hall (1974) to this system using their concept of "feeding range," which refers to the size range of food particles that can be engulfed by a mouth of any given gape. Their ideas can explain the spacing between adjacent cohorts. This interval (in effect the difference in mean body size) tends to minimize intraspecific competition by allowing each cohort a virtually independent food supply. This independence is generated by differences in mouth size. Thus, capelin cohorts are spaced closer together than are those of herring (Fig. 2 and 3) because the latter, having larger mouths then capelin, are capable of exploiting a wider

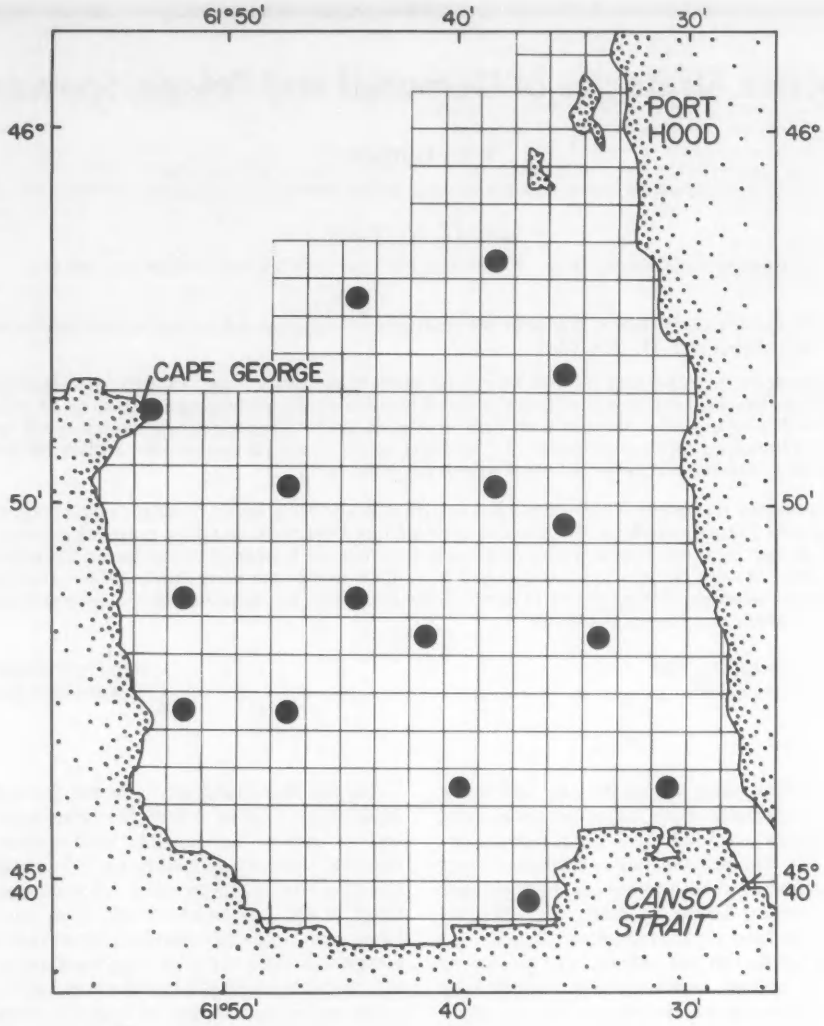

FIG. 1. Location of zooplankton sampling stations during 1974, St. Georges Bay.

range of food particles and require proportionally greater spacing between cohorts.

Upon comparing cohorts between years it became clear that spacing was also related to larval growth rate. This seems reasonable, for if we extend Jones and Hall's (1974) reasoning it is easy to see that the faster any particular "feeding slot" is vacated by a cohort, the sooner it can be filled by the next.

The data on herring and capelin larval growth and cohort interval could be fitted well to a power curve (Fig. 4). The interval between cohorts is the time period between successive releases of larvae and is determined by back-extrapolating the cohort lines to size at hatching (see Lambert 1984). A complete 4-yr series is not available for either species because low larval abundance in some years precluded any formal analysis. If this curve is extrapolated (broken line in Fig. 4) towards the right it makes an interesting prediction. As larval growth rate approaches and exceeds 1 mm/d, the interval between successive cohorts becomes vanishingly small so that, in effect, spawning can occur continuously, or all the eggs can be released at once.

Does this happen? Having measured the larval growth rate of two pelagic spawners, Atlantic mackerel and white hake, we were able to test this. The mean growth rates over the first few weeks for mackerel and hake (0.85 and 0.70 mm/d, respectively) were plotted on the extrapolated curve at the appropriate position and indicated that the spacing between larval cohorts should be 2.2 and 3.0 d for mackerel and hake, respectively. Such intervals would not be detected with the time resolution of most field sampling programs, and spawning would appear to be continuous.

The spawning behavior of mackerel and hake was examined and found to be consistent with the prediction. In both species, spawning, although detectable for about a 2-mo period, was concentrated within roughly a 2-wk span (Fig. 5), resulting in the appearance in the plankton of one huge cohort of newly hatched larvae.

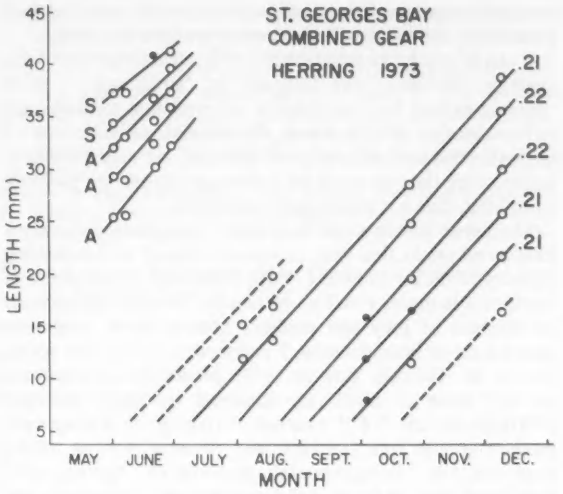

FIG. 2. Larval herring cohorts, St. Georges Bay, 1973. ○, mean size of cohort derived by polymodal population analysis; ●, mean size of cohort plotted by eye. Numbers to the right are growth rates (mm/d) of respective cohorts; S and A refer to summer and autumn cohorts from previous year.

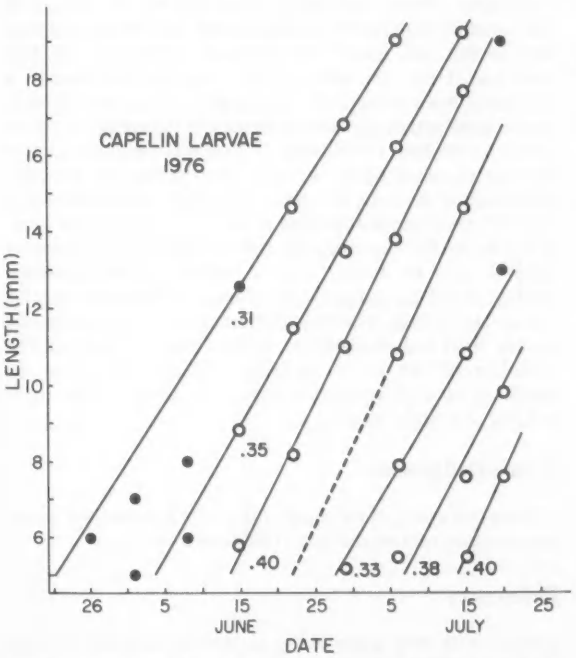

FIG. 3. Larval capelin cohorts, St. Georges Bay, 1976. ○, mean size of cohort derived by polymodal population analysis; ●, mean size of cohort plotted by eye. Numbers are growth rates (mm/d) of respective cohorts.

Thus, we have evidence of two extremes of spawning tactic with the suggestion of a continuum between. On one hand, two pelagic spawners release over a short period large quantities of eggs, which hatch into fast-growing larvae whereas, on the other, the demersal spawners deposit over a long period of time

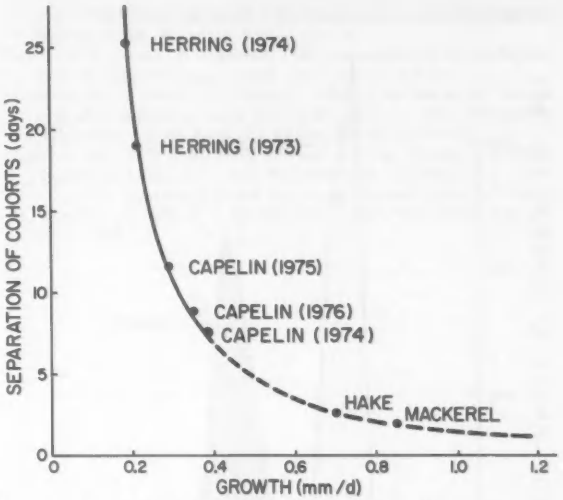

FIG. 4. Relationship between larval growth rate and interval between cohorts. Line fitted to capelin and herring points and extrapolated beyond; $y = 1.71x^{-1.58}$, $r = -0.99$, $p = 0.001$.

fairly regularly spaced batches of eggs, which hatch into slow-growing larvae.

How do these reproductive strategies relate to the two major problems facing fish larvae, i.e. finding enough to eat and avoiding being eaten. Ricker and Foerster (1948) suggested that by the end of the larval stage, slow-growing larvae will have suffered greater cumulative mortality than faster growing larvae even when both had the same instantaneous mortality rate. The cumulative mortality is generated by predators. The distribution of sizes of prey vulnerable to a predator(s) has been termed a "predatory field" by Shepherd and Cushing (1981). Thus, cumulative mortality would be determined by the speed at which larvae pass through the predatory field, the rate of passage being dependent on temperature and the abundance of food. As a rule, the instantaneous mortality rate of marine organisms is inversely proportional to their size (Ware 1975; Cushing 1975), so it would appear to be advantageous for a pelagic fish to become large as soon as possible.

Therefore, mackerel and hake have opted to flood the environment with large numbers of eggs during the time when zooplankton production is highest (Hargrave et al., Marine Ecology Laboratory, Dartmouth, N.S., manuscript). A rich food supply and warm summer water temperature allow a high growth rate, and the larvae pass quickly through their respective predatory fields. Although initial mortality may be high, the compensation is a viable level of total or cumulative mortality at the end of the larval stage.

This strategy demands very precise timing, since there must be a near synchronous release of eggs that should hatch as closely as possible to peak copepod nauplii production. Ware (1977) affirmed this by showing in St. Georges Bay that peak biomass in 80-μm mesh net samples (dominated by juvenile copepod stages) coincided with peak mackerel spawning. Thus, newly hatched mackerel have a plentiful supply of copepod nauplii to feed on. Thereafter, the mackerel larvae and their food supply grow in concert until the copepods become adult, some 2–3 wk later (Marine Ecology Laboratory 1980).

The factor that orders the sequence of events appears to be

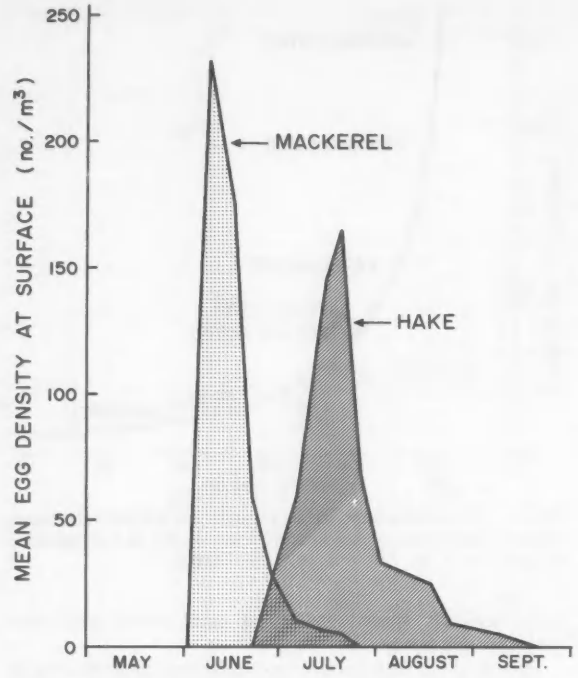

FIG. 5. Density of mackerel and white hake eggs in surface waters of St. Georges Bay, 1976. Each data point is the mean of nine stations.

water temperature. Peak mackerel spawning occurs at 13°C (SD 1.0°C) (D. M. Ware and T. C. Lambert, manuscript) and peak adult copepod biomass (2–3 wk later) at 19°C (SD 1.0°C) (Lambert et al. 1982); therefore, given the predictability of seasonal trends in St. Georges Bay, mackerel larvae will be reasonably assured of a good food supply if their parents use water temperature as a cue to trigger spawning.

In contrast, herring and capelin seem to have evolved a "bet hedging" strategy and release eggs in batches throughout the season, rather than all at once. In addition to ensuring a more equitable distribution of food, the cohort system would also be a hedge against predation. The larval growth rate of these species is low, as it must be, since many cohorts are released when food is scarce; thus, they are potentially subject to high levels of predator-inflicted cumulative mortality. Over the year, predation pressure on the succession of cohorts would be variable. Although the cumulative mortality might be very high for some cohorts, especially in the summer, there is a good chance that predation pressure on one or more cohorts would be low enough to ensure successful recruitment. It is tempting to argue that because of probable high summer mortality, summer spawning stocks historically have fared poorly in relation to spring- and autumn-spawning stocks.

One might wonder then if a continuous release of larvae might have even better adaptive value. However, there must be a maximum rate at which a predator can remove prey, and a small, continuous release of larvae into the pelagic zone could well fall below this rate, with disasterous consequences. If, on the other hand, larvae are accumulated and released in high-density batches, predator swamping can occur (Frank and Leggett 1982). In this respect the all at once strategy of pelagic spawners is very similar, for as well as ensuring that the maximum number of larval offspring encounter peak copepod production, the tactic will also lead to predator swamping.

It can be argued that the tactics employed at either end of this strategic spectrum, "bet hedging" or "all at once," are a response to both food availability and predation pressures and reflect the fact that in nature the relative contributions of starvation and predation to larval mortality are very difficult to assess, since the two rarely act independently and are probably more often than not inextricably enmeshed.

The cohort system would be adaptive, as regards food, only if total larval production were potentially limited by the carrying capacity of the environment. Until a thorough investigation of patchiness is made, conclusions reached through comparisons of numbers of prey and predator gleaned from integrative samples are at best tentative. Nevertheless, it is worth noting that in St. Georges Bay in 1977, production of copepods in July (time of maximum mackerel hatching) exceeded 1600 kg(wet)·km^{-2}·d^{-1} whereas, during spring and autumn, production was less than 800 and often as low as 400 kg (wet)·km^{-2}·d^{-1} (Hargrave et al., manuscript). Cushing (1983) concluded that haddock (*Melanogrammus aeglefinus*) and plaice (species not given) are probably too dilute during the early larval stages to affect their food, but this is probably not true for demersal spawners like herring, which have no drift phase prior to hatching, so initial densities of larvae can be high (Graham and Chenoweth 1973).

den Boer (1968), while studying carabid beetles, concluded that variation within natural populations and the environment they inhabit will result in a decrease in the risk of their extinction. Under the influence of a varying environment a population has a number of ways in which to spread this risk. One of these apparently used by herring is "spreading of risk in time." As den Boer (1968) noted, "If there is a high variation in the rate of development or time of reproduction or both, individuals of the same developmental stage will be exposed to different environmental factors at different times of the year. Hence within the same stage the risk of dying and the chance of survival may be spread over a variety of environmental conditions and consequently the chance to reproduce may be spread." In the case of herring, environmental conditions would include food and predators as well as physical factors. The spreading of risk or bet hedging strategy will reduce the amplitude of fluctuations in numbers, thereby contributing to stability of population size.

Acknowledgments

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Uptake and Accumulation of Inorganic Tin by a Freshwater Alga, *Ankistrodesmus falcatus*

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Uptake of Sn by *Ankistrodesmus falcatus* occurred very rapidly. The alga accumulated 2.3 and 4.1 mg Sn/g dry weight of cells after 2 and 60 min of incubation, respectively, in the presence of 46 μg Sn/L of medium. Increasing Sn concentrations in the medium resulted in a steady increase in the Sn concentrations in the cells. At 6 mg of Sn and 3 mg of dry weight of cells in 1 L of medium, the cells had accumulated 100 mg Sn/g dry weight of cells, representing 10% of the dry cell mass. The uptake of Sn from the medium and the release of Sn from the cells were not affected by other metals (Pb, As, Zn, Cd, Hg, Sb, and Cu). Addition of EDTA, however, caused an immediate and rapid release of 60% of Sn from the cells. Heat-killed cells took up Sn faster and to a higher level than living nonheated cells. About 85% of Sn taken up by the cells was recovered in the cellular polysaccharide fraction and 15% in the protein fraction, while the lipid and the small molecular weight metabolites accounted for 0.2% of the total Sn.

L'absorption de Sn par *Ankistrodesmus falcatus* se produit très rapidement. L'algue avait accumulé 2,3 et 4,1 mg de Sn par gramme de cellules sèches après 2 et 60 min d'incubation respectivement en présence d'un milieu contenant 46 μg Sn/L. L'augmentation des concentrations de Sn dans le milieu a conduit à une augmentation régulière des concentrations de Sn dans les cellules. À 6 mg de Sn et à 3 mg de cellules desséchées dans 1 L de milieu, les cellules avaient accumulé 100 mg Sn/g ce qui représente 10 % de la masse de cellules sèches. L'absorption de Sn du milieu et sa libération par les cellules ne touchait pas les autres métaux (Pb, As, Zn, Cd, Hg, Sb et Cu). L'addition d'EDTA toutefois produisait une libération immédiate et rapide de 60 % du Sn des cellules. Les cellules tuées par la chaleur absorbaient le Sn plus vite et jusqu'à un maximum plus élevé que les cellules vivantes non chauffées. Environ 85 % du Sn absorbé par les cellules était récupéré dans la fraction polysaccharide des cellules et 15 % dans la fraction protéinique, tandis que les lipides et les métabolites de faible masse moléculaire ne contenaient que 0,2 % du Sn total.

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Increased industrialization has resulted in increased Sn contamination of the aquatic environment. Tin is widely used in plating, solders, alloys, food cans, toothpaste, and industrial processes such as glass production and textile printing (Piscator 1979). Although there are a few reported measurements of Sn contamination in the environment (Iverson and Brinckman 1978; Seidel et al. 1980; World Health Organization 1980), little information exists on the pathway of Sn, especially on the uptake of Sn by microorganisms (Rai et al. 1981). Attramadal and Svatun (1980) reported that the uptake of Sn by bacteria was independent of cell metabolism but was influenced by the pH of the medium. Ishii (1982) observed that inorganic Sn compounds were more readily accumulated than organic Sn compounds by marine algae. To our knowledge, no other information is available on the uptake of Sn by freshwater algae, in spite of the importance of algae in aquatic food chains.

Our laboratory is concerned with the contamination, toxicity, and pathway of Sn in the aquatic environment. We have developed sensitive analytical techniques to detect the occurrence of various Sn compounds (Maguire and Huneault 1981; Chau et al. 1982; Maguire and Tkacz 1983; Maguire et al. 1982; Maguire 1984), studied the toxicity and methylation of Sn compounds (Wong et al. 1982; Chau et al. 1981), and examined the degradation of butyltin species in water (Maguire et al. 1983) and in *Ankistrodesmus falcatus* (Maguire et al. 1984). In the present study, we report the ability of *A. falcatus* to accumulate inorganic Sn several thousand times above the ambient concentration in the medium.

Materials and Methods

The axenic culture of *Ankistrodesmus falcatus* var. *acicularis* (Ontario Ministry of the Environment, Rexdale, Ont.) was grown in 100 mL of CHU-10 medium at pH 8 (Chu 1942) at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light ($6\ \mu E \cdot m^{-2} \cdot s^{-1}$) and 6 h of darkness. When the culture reached the logarithmic phase of growth (1 wk), it was separated from the medium by centrifugation at 16 000 × *g* for 20 min. No significant cell breakage was found from the centrifugation. Fresh medium (100 mL) was then used to wash the culture once to remove excretory products from the cells, which were then resuspended in 50 mL of fresh medium for experimental use.

Tin was added as $SnCl_4$ with carrier-free ^{113}Sn as a tracer (^{113}Sn in 6 mol/L HCl from Amersham Corp.). Tin dilution was made with CHU-10 medium and adjusted to pH 8. Total Sn in the cells and in the medium was monitored by direct measurement of ^{113}Sn and calculated by the ratio of ^{113}Sn and unlabelled Sn.

To determine the time course of Sn uptake, 50 mL of cell suspension in a 250-mL Erlenmeyer flask was spiked with 0.5 mL of $SnCl_4$ (370 kBq of ^{113}Sn and 4.6 μg Sn/mL of stock solution). The flask was thoroughly mixed in a shaker. Five millilitres of the cell suspension was withdrawn after 2, 5, 10, 15, 30, 45, 60, 120, and 240 min of incubation and was filtered through a membrane filter (Sartorius) with 0.45-μm pore size and rinsed rapidly with 10 mL of fresh CHU-10 medium to remove extracellular radioactivity. To compensate for radioactivity adhering to the filter, an equal volume of medium (without the cells) and ^{113}Sn was filtered and washed. The radioactivity representing less than 7% of the amount taken up by the cells was subtracted. The radioactivity was measured with an automatic well-type gamma scintillation counter (Nuclear Chicago) with 43% counting efficiency for ^{113}Sn. The concentration factor was the ratio between the concentrations of ^{113}Sn in the cells and in the medium. Cell dry weight was obtained by filtering and drying the algal cells at 60°C for 48 h. A correction was made for the dry weight of the CHU-10 medium. Five millilitres of cell suspension contained 20 μg cell dry weight or 2.2×10^5 cells.

Five millilitres of washed cell suspension (approximately 15 μg dry weight) was exposed to $SnCl_4$ solution at concentrations varying between 46 μg and 6 mg Sn/L. Fifty microlitres of ^{113}Sn (370 kBq/mL) was added as radioactive tracer. The cells were incubated in the shaker at 20°C under light for 90 min. They were then filtered, washed, and counted as described.

To investigate effects of other metals on uptake and retention, a batch of washed cells was divided into two parts. One part was used for the measurement of ^{113}Sn uptake and the other for ^{113}Sn retention by *A. falcatus* in the presence of other metals. To measure ^{113}Sn uptake, 5 mL of cell suspension was incubated with 2.5 μg of unlabelled Sn and 18 kBq of radioactive Sn (final concentrations of 0.5 mg and 3.7 MBq/L) in the presence of 0.5 or 3.0 mg Pb, As, Zn, Cd, Hg, Sb, or Cu (as chloride salts)/L. After 90 min of incubation, the cells were filtered, washed, and counted as described. To measure ^{113}Sn retained, cells previously labelled with ^{113}Sn, as described, were centrifuged, washed, and resuspended in a fresh medium with or without the presence of 0.5 or 3.0 mg of the various test metals/L. After 90 min of incubation, the cells were filtered, washed, and counted.

The time-course of ^{113}Sn uptake in "live" and "dead" cells was followed as described. One hundred millilitres of cell suspension was divided into two equal parts. One part was placed in hot water (90°C) for 10 min. To compensate for evaporation, the volume was brought back to 50 mL with medium and the pH was adjusted to 8. No viable cell was found when a small aliquot was plated onto a CHU-10 agar plate. This part was designated as "dead" cells. The other part was left untreated and labelled as "live" cells.

The effects of EDTA on Sn release were studied by filtering 5 mL of each of the live and dead cell suspensions after 1, 5, 10, 20, 30, 40, 50, and 60 min of incubation. The remaining cell suspensions were divided into two parts. One part received a small volume of concentrated EDTA (final concentration of 1 mmol/L), while the other portion received an equivalent volume of medium. Five millilitres of cell suspensions with EDTA was filtered after 5, 10, 20, 40, 70, and 90 min of EDTA addition. Five millilitres of cell suspensions without EDTA addition was filtered after 60 and 100 min.

Incorporation of Sn in cellular components was investigated with triplicate 100-mL samples of washed cell suspensions (approximately 1.5 mg cell dry weight) incubated with labelled and unlabelled Sn (110 kBq and 0.2 mg) for 4 h at 20°C under light. At the end of incubation, the cells were filtered through a glass-fiber Whatman filter (0.7 μm) and washed with 100 mL of medium. The cells were then fractionated with chloroform–methanol and trichloroacetic acid according to the procedures of Li et al. (1980).

Results

When *A. falcatus* was exposed to the medium containing 46 μg Sn/L, the alga took up the metal from the medium rapidly (Fig. 1). At the first sampling time of 2 min, the Sn concentration in the algal cells reached 2.3 mg/g dry weight of cells. The Sn concentration factor in the cells and in the medium was calculated to be 5×10^4. Further incubation showed a steady increase of Sn in the cells. There was no significant increase after 60 min of incubation. At this steady state, the cells contained 4.1 mg Sn/g dry weight of cells and had a concentration factor of 8.9×10^4.

The ability of *A. falcatus* to accumulate the Sn compound was further tested in the presence of increasing amounts of Sn in the medium (Fig. 2). At 90-min exposure time (steady state), the alga showed a remarkable ability to bind Sn. There was a linear relationship between Sn in the cells and Sn in the medium when the concentration of Sn in the medium was below 1 mg/L. At the highest concentration of 6 mg/L in the medium, the cells accumulated 100 mg Sn/g of cells, representing 10% of the dry cell mass. Since 1 g of cells comprised 1.1×10^{10} cells, the amount of Sn in the alga was equivalent to about 4.6×10^{10} Sn atoms per cell.

The effects of other metals (Pb, As, Zn, Cd, Hg, Sb, and Cu) on the uptake and release of Sn from the cells were tested. The metals at 0.5 and 3.0 mg/L did not significantly interfere with the uptake of Sn by the cells nor did they induce the release of Sn from the cells (Table 1).

The uptake of Sn by "live" (nontreated) and "dead" (heat-killed) cells was followed in a time-course study. Both types of cells showed a similar pattern in the Sn uptake (Fig. 3). The dead cells had a faster rate and a higher steady state of Sn accumulation than the live cells.

The live and dead cells were allowed to accumulate Sn until the steady-state level was reached. At this time (60 min), half the cells received EDTA (final concentration 1 mmol/L) and the

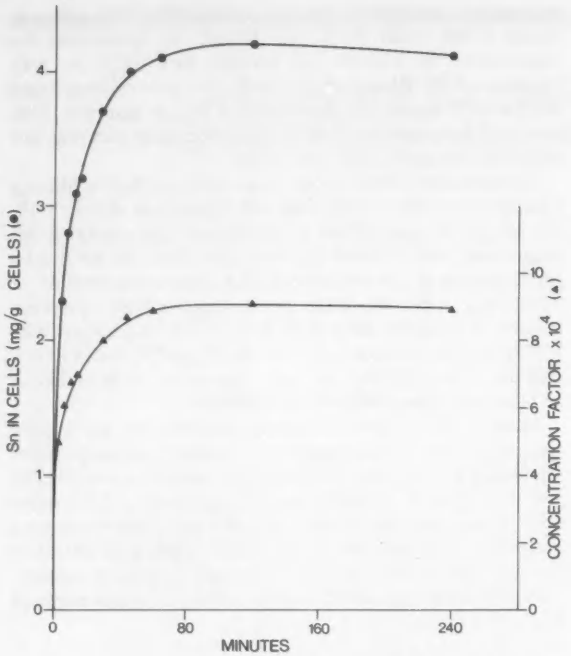

FIG. 1. Time-course of Sn uptake and accumulation by *A. falcatus*. Radioactivities were determined after 2, 5, 10, 15, 30, 45, 60, 120, and 240 min of incubation (concentration factor scale 20 000 – 100 000).

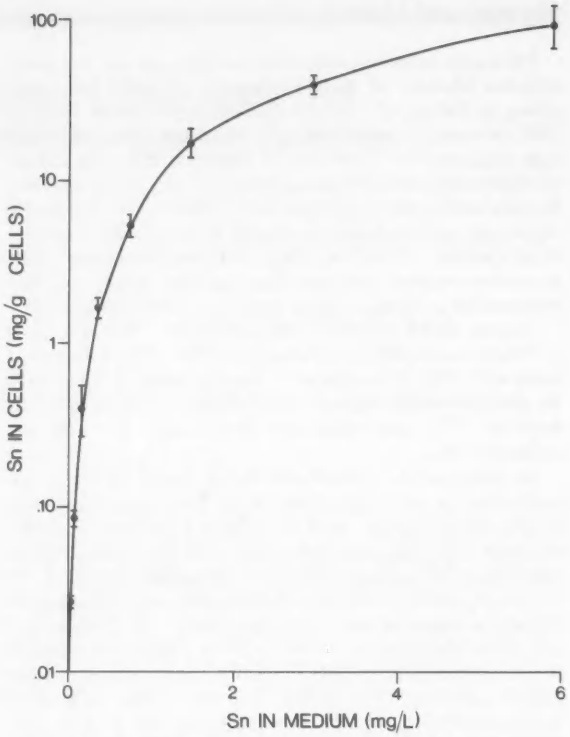

FIG. 2. Relationship between Sn concentrations in the medium and that in cells. The radioactivities accumulated in cells were measured in relation to its concentration in medium after 90 min of incubation.

other half received an equivalent volume of the medium (see Materials and Methods). As shown in Fig. 4, the addition of EDTA caused an immediate and rapid release of 60% of Sn from both live and dead cells, while the controls with the addition of the medium did not release the Sn. New levels (40%) of Sn in the cells were established in 20 min after the EDTA addition. These new levels were similar to the levels of the cell uptake in 2 min. Interestingly enough, the dead cells again retained a higher Sn level than the live cells.

Ankistrodesmus falcatus was exposed to Sn for 4 h and the algal cells were fractionated into several components: lipid, protein, small molecular weight metabolites, and polysaccharide. About 85% of the Sn in the cells was in the polysaccharide fraction, while 15% was in the protein fraction (Table 2). Lipid and small molecular weight metabolites contained a negligible amount of Sn.

Discussion

It is well known that algal species can accumulate high concentrations of metal from water (Schulz-Baldes and Lewin 1976) and from sediment (Laube et al. 1979). Such accumulation by algae can result in the movement of metals up the food chain to higher trophic levels. At the very low concentration of Sn in the medium (46 μg Sn/L), *A. falcatus* accumulated Sn to a concentration factor of 9.1×10^4 (Fig. 1). The uptake of Sn from the medium by this alga appeared to occur in two phases: a rapid uptake in the first 15 min and a slower increase thereafter. This curvilinear relationship (an initial linear uptake that levels off, becoming asymptotic with the duration of exposure) is generally observed in metal uptake by various organisms. The initial uptake is believed to be related to the adsorption of metal

TABLE 1. Effects of metals on the uptake and retention of Sn by *A. falcatus*.

Addition of metal	Concentration (mg/L)	% of metal	
		Uptake	Retention
None (control)[a]	—	100[b]	100[c]
Pb	0.5	102	98
As	0.5	89	103
Zn	0.5	111	102
Cd	0.5	92	100
Hg	0.5	86	97
Sb	0.5	89	98
Cu	0.5	94	104
Pb	3.0	132	101
As	3.0	102	99
Zn	3.0	106	104
Cd	3.0	108	99
Hg	3.0	111	99
Sb	3.0	99	94
Cu	3.0	115	90

[a]Sn concentration in the medium was 0.5 mg/L.
[b]100% uptake of Sn in the absence of other metal represented 9.5 mg Sn/g dry weight cells.
[c]100% retention of Sn in the absence of other metal represented 11.5 mg Sn/g dry weight cells.

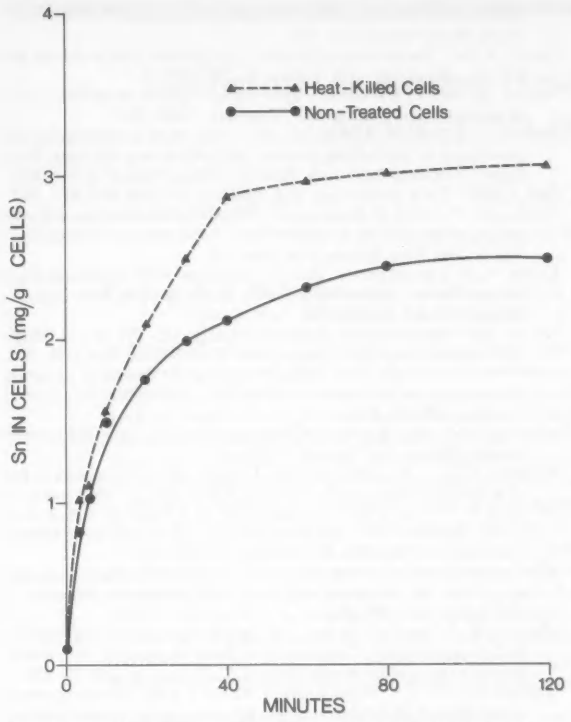

FIG. 3. Uptake of Sn in heat-killed cells and nontreated cells at various incubation times.

TABLE 2. Distribution of ^{113}Sn in cellular components of *A. falcatus*.

Cellular component	Counts/min[a]	%[b]
Lipid	28±4	0.1
Small molecular weight metabolites	52±7	0.1
Protein	6 750±193	14.8
Polysaccharide	38 703±2010	85.0

[a]Mean ± SD of three replicates.
[b]Sum of total radioactivities in the cellular components representing 45 533 counts/min or 100%.

to the cell surface, followed by a slower passage through the cell membrane (Fujita and Hashizume 1975; Horowitz and Presley 1977; Rai et al. 1981).

The ability of *A. falcatus* to accumulate Sn was further demonstrated when the Sn concentration in the medium was increased to 6 mg/L. At such a concentration, the cells accumulated Sn to a level equivalent to 10% of the dry cell mass (Fig. 2). Attramadal and Svatun (1980) observed that a bacterium could accumulate Sn even to 18% of the cellular dry mass. Pribil and Marvan (1976) demonstrated that the alga *Scenedesmus quadricauda* could accumulate uranium to 15–17% of the dry weight of the cells. While there is a general agreement in the literature on the ability of microorganisms to accumulate metals, there is still controversy concerning the competition between metals during uptake. For example, Sakaguchi et al. (1979) found that the adsorption of Cd by *Chlorella* cells was inhibited by other cations, while Stokes (1975) reported that Cu increased the uptake of Ni by *Scenedesmus*. The lack of competition between Sn and other cations in

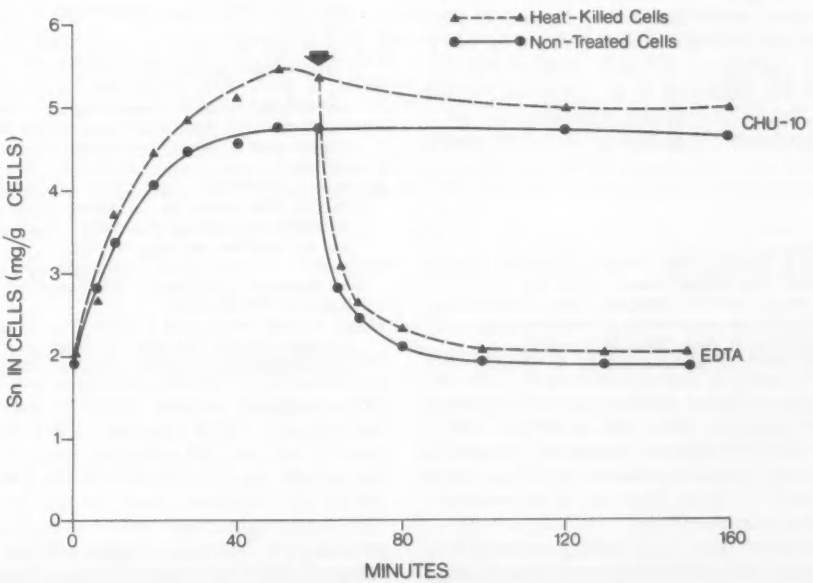

FIG. 4. Effects of EDTA on Sn released from the cells.

A. falcatus in our study (Table 1) suggested that either Sn had a higher affinity for the same binding sites or the Sn and other cations had different binding sites in the cells.

The uptake of Sn from the medium by *A. falcatus* appeared to be strictly by physicochemical surface adsorption and not by cell metabolism. This was shown by the uptake of Sn in the live and dead cells (Fig. 3). The dead cells took up more Sn than the live cells. The heat treatment of the cells could increase the negative charge of the cells or increase the membrane permeability for Sn to bind the intracellular organelles. The uptake of Sn by a bacterium and the uptake of Cd by an alga were also higher in the dead cells than the live cells (Attramadal and Svatun 1980; Sakaguchi et al. 1979). The cell-bound Sn could not be released by other cations (Table 1) but 60% was removed by EDTA (Fig. 4). As EDTA was incapable of penetrating the cell membrane of living cells (Attramadal and Svatun 1980), the results would suggest that the major part of the bound Sn was located outside the cell membrane in the live cells. In the dead cells, EDTA should be able to penetrate the denatured cell membrane and should cause more Sn to be released from the cells. However, the same proportional amount of Sn was displaced by ADTA in both live and dead cells. This suggested that about 40% of the Sn was very strongly bound to the cells and could not be displaced by EDTA.

The nature of the binding sites for Sn is believed to be the phosphate groups in the cell wall and cell capsule of a bacterium (Attramadal and Svatun 1980). Since polysaccharides are important constituents of cell wall and cell membranes of various algae, they are the preferred sites for metal binding (Veroy et al. 1980; Rai et al. 1981). Our results indicated that about 85% of the Sn taken up by *A. falcatus* was recovered in the polysaccharide fraction (Table 2). Another alga, *Scenedesmus* sp., was reported to contain 80% of the vanadium in the polysaccharide fraction (Lee 1983). The polysaccharide, carrageenan, in two marine algal species bound Pb and Cd from the water (Veroy et al., 1980).

In summary, *A. falcatus* was able to accumulate Sn rapidly from the medium. The uptake and release of Sn from the cells were independent of cell metabolism and the presence of other metals but were reduced by EDTA. The majority of Sn was in the polysaccharide fraction of the cells. Whether Sn was concentrated in the cell surface or in the cytoplasm was not known. However, this is not important in consideration of its transfer to higher trophic levels, as both forms may be equally available.

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Multistage Sampling Designs in Fisheries Research: Applications in Small Streams

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A common, although generally unrecognized, use of multistage sampling designs in freshwater fisheries research is for estimation of the total number of fish in small streams. Here there are two stages of sampling. At the first stage one selects a sample of stream sections, usually of equal length, and at the second stage one estimates the total number of fish present in each selected section. This paper argues that the conventional practice of selecting stream sections of equal length is ill-advised on both biological and statistical grounds, and that errors of estimation of fish numbers within selected sections will usually be small compared with errors of estimation resulting from expansion of sampled sections to an entire stream. If stream sections are instead allowed to vary in size according to natural habitat units, then alternative two-stage sampling designs may take advantage of the probable strong correlation between habitat unit sizes and fish numbers. When stream sections of unequal sizes are selected with probabilities proportional to their size (PPS), or measures of the sizes of selected sections are incorporated into estimators, one may substantially increase precision of estimation of the total number of fish in small streams. Relative performances of four alternative two-stage designs are contrasted in terms of precision, relative cost, and overall cost-effectiveness. Choice among alternative designs depends primarily on the correlation between fish numbers and habitat unit sizes, on the total number of stream sections, and on sample size. Recommendations for choices among the designs are presented based on these criteria.

Les plans d'échantillonnage à étapes multiples, servent souvent, sans toutefois être généralement reconnus, dans le domaine de la recherche sur les pêches en eau douce pour estimer le nombre total de poissons dans les petits cours d'eau. Ici, l'échantillonnage se fait en deux étapes. Dans un premier temps, on choisit un échantillonnage de sections d'un cours d'eau, habituellement de longueurs égales puis, dans un deuxième temps, on estime le nombre de poissons présents dans chacune des sections choisies. Le présent document avance que la méthode voulant qu'un choisisse des sections du cours d'eau de longueurs égales est mal inspirée tant du point de vue biologique que statistique et que les erreurs que comporte l'estimation du nombre de poissons dans les sections choisies seront généralement petites comparativement à celles résultant de l'extrapolation des résultats à la totalité du cours d'eau. Si, au contraire, on fait varier la longueur des sections en fonction des habitats naturels, alors les plans d'échantillonnage à deux étapes pourront tenir compte de la forte corrélation qui existe probablement entre la taille de l'habitat et le nombre de poissons. Il est possible d'augmenter de façon substantielle la précision du calcul du nombre total de poissons dans les petits cours d'eau en choisissant des sections du cours d'eau de longueurs inégales présentant une probabilité à leur taille (PPT), ou en incorporant dans les estimateurs les mesures relatives à la taille des sections choisies. On compare la performance relative de quatre plans à deux étapes pour ce qui est de la précision, du prix de revient relatif et du rendement global en termes de coût-efficacité. Le choix du plan dépendra surtout de la corrélation entre le nombre de poissons et la taille de l'habitat, du nombre total de sections du cours d'eau considérées et de la taille de l'échantillon. En se basant sur ces critères, on a formulé des recommandations sur la façon de choisir le plan à utiliser.

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Many applications of statistical survey (sampling) theory in marine fisheries research have involved use of multistage sampling designs (Abramson 1968; Tomlinson 1971; Southward 1976; Schweigert and Sibert 1983). In multistage sampling there are two or more levels (stages) of sample selection. For example, suppose one wished to estimate the mean length of a commercial fish species landed at a particular port (over some specified period of time). The first stage of sampling might be selection of a particular sample of fishing vessels which lands catch of that species at that port. Given a first stage selection of vessels, one must sample from the vessels' catches. If fish are unloaded in large bins, then one selects a particular sample of bins at the second stage of sampling (from each of those vessels selected at the first stage). If the number of fish within selected bins is large, then subsampling of bins may be required; within each selected bin a third stage sample of fish may be drawn and on these fish actual measurements of fish length would be made.

In the simplest case, equal probability selection methods are used at each stage of sampling: each vessel entering a port has an equal chance of inclusion at the first stage, each bin from a selected vessel has an equal chance of inclusion at the second

stage, and each fish within a selected bin has an equal chance of being measured at the third stage. Either simple random sampling (SRS) or systematic sampling could be used at each stage, although systematic sampling (e.g. one in every k vessels) may often prove to be more practical. Each stage of sampling therefore requires choice of selection method. These choices determine which multistage estimators are appropriate and may, in general, strongly influence the variance and/or bias of resulting estimates (here, of mean length). The term multistage sampling design is used to define a specific collection of selection procedures (used at each stage of sampling) and their associated estimators of attributes and of the variances of those estimated attributes.

One must also choose the sample sizes at each stage of selection; in sampling theory jargon, one must choose the sampling fractions at each stage. What fraction of the vessels should be included at the first stage? What fraction of a selected vessel's bins should be included at the second stage? What fraction of those fish within selected bins should be measured? Providing answers to these kinds of questions has often been the principal focus of papers devoted to the use of multistage sampling designs in fisheries research (Schweigert and Sibert 1983; also, Cuff and Coleman 1979 for an example using stratified sampling). Given estimates of variance at each stage of sampling, one may determine optimal sampling fractions at each stage based on criteria of (a) minimizing the variance of resulting estimates at fixed cost or (b) achieving a specified variance at minimum cost (see Cochran 1977, p. 313–316).

Less attention has been given to selection method per se or to the use of auxiliary variables in fisheries research (see Lenarz and Adams 1980 for a comparison of SRS, systematic, and stratified SRS in groundfish trawl surveys). Unequal probability selection methods, which often rely on information provided by some inexpensively gathered auxiliary variable, seem especially worthy of attention. In the preceding example, selection of vessels according to (the auxiliary variable) vessel size may increase the precision of resulting estimates and do so in a cost-effective manner. Because the number of fish landed may be highly correlated with a vessel's size, landings from a large vessel will usually have more influence on mean fish length than will landings from a small vessel. By setting first stage selection probabilities proportional to a measure of vessel size (PPS), one creates a selection procedure whereby larger vessels are more likely to be included in first stage selections than are smaller vessels (see Tomlinson 1971). SRS is a special case of such unequal probability sampling (Kendall and Stuart 1983, p. 189–195).

Multistage sampling designs have seen less frequent use in freshwater fisheries research. However, a very common, although generally unrecognized, use is for estimation of the total number of fish in small streams. In this context there are two stages of sampling. At the first stage, a particular set of stream sections, usually of equal length, is selected (usually by SRS). Within any selected stream section, some population estimation techniques, most frequently a removal method estimator (Seber 1982, Sect. 7.2) based on electrofishing, is used to estimate the total number of fish present. Because there are no discrete sampling units at the second stage of sampling (bins were obvious discrete sampling units in the commercial fishery example), multistage sampling theory has not been previously used to explicitly address estimation of the total number of fish in small streams. However, one may make an analogy between (a) estimating the total number of fish present in a stream section (a first stage or primary unit) by electrofishing and (b) estimating the mean length of fish from a particular vessel by subsampling of a vessel's bins.

To make this analogy obvious, assume that there are only two stages of sampling for the commercial fishery example: all fish within selected bins are measured. Then, the bounds of error of estimation of mean fish length from a particular vessel arises from sampling variance: the variation among all those possible estimates of mean fish length derived from all of the possible samples (finite in number) of n bins selected from the N bins on that vessel. Because this variation results at the second stage of sampling, it is termed second stage variance (within a selected primary unit). When electrofishing is used to estimate the total number of fish in a selected primary unit (the primary unit total), second-stage variance depends on the variance of the sampling distribution of the removal method estimator. This sampling distribution may be visualized as a plot of the relative frequency of particular estimates of the primary unit total. However, to obtain such a plot one must imagine repeating the electrofishing/removal method process an infinite number of times, while assuming a known primary unit total and a known and constant capture probability (the chance that a fish which is present at the time of electrofishing will be captured by electrofishing). Determination of second stage variance for this kind of sampling distribution, in contrast with the case of sampling from a discrete number of bins, requires construction of a formal stochastic model and use of specific probability distribution assumptions (Seber 1982, Sect. 7.2; Schnute 1983).

Bohlin (1981) recently treated the usual two-stage stream survey problem in terms of sources of error: (a) error arising from variation among (estimated) primary unit totals and (b) "measurement" error within selected primary units. The variance formulae he derived are equivalent to those presented by Raj (1968, p. 116–119) and Cochran (1977, p. 300–303) for two-stage sampling of equal-sized primary units if electrofishing sampling variance is equated with second stage variance. However, Bohlin did not view this problem in terms of multistage sampling theory.

In this paper it is argued that (a) the usual practice of selecting sections of equal length is ill-advised on both biological and statistical grounds and that (b) a preferable practice is to allow stream sections to vary in size according to natural habitat units. If primary units (stream sections) are of unequal sizes, then several alternative two-stage sampling designs may be used in the context of stream surveys. In particular, (a) primary units may be selected with probabilities proportional to primary unit sizes and (b) the use of an auxiliary variable (here, primary unit size) may result in substantial improvements in the precision of estimation of the total number of fish in small streams. Average costs for alternative designs may be computed and the cost-effectiveness of alternative designs compared. Although alternative selection procedures and the benefits of using auxiliary variables will be demonstrated in the narrow context of small stream surveys, the general principles and procedures used should be applicable in a broad range of fisheries contexts, both freshwater and marine.

Sampling Designs

Notation and definitions for all sampling designs presented in this paper are summarized below:

Notation	Definition
N	total number of primary units in the sampling universe
n	total number of primary units in the sample
Y_i	total in primary unit i; estimated as $\hat{Y}_i$
$Y = \sum^{N} Y_i$	total across all primary units
$\bar{Y} = Y/N$	mean of all primary unit totals; estimated as $\hat{\bar{Y}} = \sum^{n} \hat{Y}_i/n$
σ_i^2	second stage variance within primary unit i; estimated as $\hat{\sigma}_i^2$
M_i	size of primary unit i
$M_0 = \sum^{N} M_i$	total size of all primary units
$\bar{Y}_i = Y_i/M_i$	mean per unit of size in primary unit i; estimated as $\hat{\bar{Y}}_i = \hat{Y}_i/M_i$
$\bar{\bar{Y}} = Y/M_0$	overall mean per unit of size; estimated as $\hat{\bar{\bar{Y}}} = \sum^{n} \hat{Y}_i \Big/ \sum^{n} M_i$
$p_i = M_i/M_0$	probability of selecting the ith unit on a given draw when units are selected by PPS with replacement
π_i	probability that the ith primary unit is in a sample of size n drawn from N by PPS without replacement
π_{ij}	probability that both primary units i and j are in a sample of size n drawn from N by PPS without replacement

An estimate is in all cases distinguished from a true value by a circumflex above. For variances, $\hat{V}(\hat{Y})$ distinguishes a sample-based estimator of variance from the true sampling variance of the estimated total, $V(\hat{Y})$. Similar notation is used to distinguish sample-based estimators and true mean square error (= variance + bias^2). Unless otherwise specified, summations are implicitly over primary units in the sample ($i = 1, 2, \ldots, n$) or in the sampling universe ($i = 1, 2, \ldots, N$). The sampling universe consists of the total number of primary units and their respective attributes from which the sample is drawn by some selection method.

The Usual Case: Primary Units of Equal Sizes Selected by SRS

If primary units of equal sizes are selected by SRS, a removal method estimator is used to estimate selected primary unit totals, and sampling variance of the removal method estimator is equated with second stage variance, then (Cochran 1977, p. 300–303; Raj 1968, p. 116, 119):

$$(1) \quad \hat{Y} = \frac{N}{n} \sum^{n} \hat{Y}_i$$

$$(2) \quad V(\hat{Y}) = \frac{N(N-n) \sum^{N} (Y_i - \bar{Y})^2}{n(N-1)} + \frac{N \sum^{N} \sigma_i^2}{n}$$

$$(3) \quad \hat{V}(\hat{Y}) = \frac{N(N-n) \sum^{n} (\hat{Y}_i - \hat{\bar{Y}})^2}{n(n-1)} + \frac{N \sum^{n} \hat{\sigma}_i^2}{n} .$$

The first term in equation 2 accounts for variation among primary unit totals (first stage variance), whereas the second term accounts for variation arising from (sub)sampling within selected primary units (second stage variance). Total sampling variance is the sum of first and second stage variances. When unbiased estimators are used at the second stage of sampling, equations 1–3 give exact and unbiased results. However, because removal method estimation of a primary unit total ($\hat{Y}_i$) is only asymptotically unbiased, and estimators for the variance of an estimated total ($\hat{\sigma}_i^2$) are only approximate (Appendix A), equations 1 and 3 are also only approximate. Equations 1 and 3 are equivalent to formulas derived by Bohlin (1981).

Although the above formulas have (approximate) statistical validity, there are serious biological problems with the convention of selecting primary units of equal sizes. If primary units are equal-length sections of stream, then a selected unit may contain more than one habitat type, and only portions of discrete, natural habitat units (e.g. portions of a pool and/or of a riffle) may be included in a selected unit. For removal method estimation using electrofishing, one delimits the primary unit boundaries by first placing block nets at the upstream and downstream ends of the selected stream section. Placement of these block nets may result in considerable displacement of fish from the primary unit to be sampled when the ends of a selected unit fall, say, midway in a pool or riffle. Also, it will sometimes be physically impossible to place block nets at the selected locations: a pool may simply be too deep. Such nonsampling error may seriously bias estimation of primary unit totals and may seriously compromise the validity of any further inferences.

There are at least two obvious ways by which to circumvent the above problems. Having identified the selected unit and having recognized that the upper end of the unit passes through the deepest part of a pool, say, one could "move the section upstream" until the entire pool, or most of it, was included. Because this alternative involves a purposive action, it destroys both the intent and statistical validity of any primary unit selection method. Alternatively, one could at least ensure that only a single habitat type would be included in any selected primary unit. If a stream were mapped and then stratified by habitat type into, say, pools and riffles, then these two strata could be independently sampled. However, this alternative would not eliminate those nonsampling errors identified above: a section could still fall midway within a pool or riffle.

If primary units were instead allowed to vary in size according to the sizes of natural habitat units, then several advantages would result. First, those nonsampling errors identified above would be minimized. Placement of block nets at the upper and lower ends of entire pools or riffles would be far less likely to displace significant numbers of fish from the unit to be sampled. Second, the numbers and sizes of fish estimated to be present in particular pools or riffles could be related to the sizes of these natural habitat units. Finally, when primary units are of unequal size, multistage sampling theory offers a variety of alternative two-stage designs with which to estimate the total number of fish in small streams.

The remainder of this paper is devoted to a presentation of four such alternative sampling designs and to a consideration of their advantages, limitations, and costs. It is assumed that stream habitat has first been mapped and stratified into habitat strata. The four sampling designs thus deal only with estimation of the total number of fish within a particular habitat stratum. Because all stratum estimates are independent of one another,

this introduces no complications or restrictions to the procedures presented and contrasted. Given independent estimates of the total number of fish in the hth stratum, $\hat{Y}_h$, and associated sample-based variances for the estimated stratum totals, $\hat{V}(\hat{Y}_h)$, estimates of stratum totals and variances are strictly additive (Raj 1968, p. 123):

$$\hat{Y} = \sum^{L} \hat{Y}_h;\ \hat{V}(\hat{Y}) = \sum^{L} \hat{V}(\hat{Y}_h);\ h = 1, 2, \ldots, L.$$

In the following, all stratum-specific notation has been avoided; notation follows that presented in the preceding summary of notation and definitions.

Alternative Designs: Primary Units of Unequal Sizes

The four alternative two-stage designs, all based on primary units of unequal sizes, may be classified by selection method and by their use (or lack of use) of an auxiliary variable (here, primary unit size):

Design A: SRS — no auxiliary variable used

Design B: SRS/ratio estimation — uses an auxiliary variable

Design C: PPS — sampling with replacement

Design D: PPS — sampling without replacement

For both PPS designs, first stage selection probabilities are based on the auxiliary variable, primary unit size. It is assumed for all designs that (a) primary unit totals are estimated by the removal method based on electrofishing and that (b) electrofishing sampling variance is equated with second stage variance. Note, however, that other estimators of primary unit totals, e.g. mark–recapture estimators, could be used at the second stage of sampling. All formulas would remain valid.

Design A: SRS

If primary units of unequal sizes are selected by SRS, but one does not employ an auxiliary variable, then equations 1–3 are again appropriate (Cochran 1977, p. 300–303). If one had unbiased estimators at the second stage of sampling, equations 1 and 3 would again be exact and unbiased.

When primary unit totals are highly correlated with primary unit sizes, first stage variance will be large for this design. In this case, individual primary unit totals, Y_i, will be very different from the average primary unit total, $\bar{Y}$, and the squared differences between Y_i and $\bar{Y}$ will make the first term in equation 2 large.

Design B: SRS/ratio estimation

If primary units of unequal sizes are selected by SRS and one also incorporates a measure of the sizes of selected primary units (M_i) in estimators, then a two-stage ratio estimator may be used (Cochran 1977, p. 300–305):

$$(4)\quad \hat{Y} = M_0 \sum^{n} \hat{Y}_i \Big/ \sum^{n} M_i$$

$$(5)\quad \mathrm{MSE}(\hat{\bar{Y}}) \approx \frac{N(N-n)\sum^{N} M_i^2(\bar{Y}_i - \bar{\bar{Y}})^2}{n(N-1)} + \frac{N\sum^{N} \sigma_i^2}{n}$$

$$(6)\quad \widehat{\mathrm{MSE}}(\hat{\bar{Y}}) = \frac{N(N-n)\sum^{n} M_i^2(\hat{\bar{Y}}_i - \hat{\bar{\bar{Y}}})^2}{n(n-1)} + \frac{N\sum^{n} \hat{\sigma}_i^2}{n}.$$

Because ratio estimation is biased, mean square error is used as a measure of precision rather than variance. However, use of the auxiliary variable, M_i, in equations 4–6 may dramatically increase precision of estimation over equations 1–3 when (a) primary unit totals are proportional to primary unit sizes and (b) the variation in primary unit totals increases in proportion to primary unit size. In biological terms, condition (a) means that the total number of fish in a selected habitat unit should be proportional to the size of the habitat unit; the larger a pool, the greater the number of fish. When the above conditions are (exactly) met, the ratio estimator is the best linear unbiased (BLUE) estimator. When the above conditions are approximately met, then the first term in equation 5 becomes small because the average number of fish per unit of habitat size within any selected primary unit ($\bar{Y}_i$) is a very stable quantity, nearly independent of primary unit size, and is approximately equal to the average number of fish per unit of habitat size in the sampling universe ($\bar{\bar{Y}}$). However, Cochran (1977, p. 162–164) noted that sample-based estimates of mean square error (equation 6) may have serious negative bias when the number of selected primary units is small ($n < 12$). Equations 5 and 6 are both large-sample approximate results.

Design C: PPS — Sampling with replacement

For this design, primary units are selected with replacement with probabilities $p_i = M_i/M_0$. Raj (1968, p. 119–121) derived estimators for this case that require independent sampling of a primary unit if it is selected more than once in the sample; no single estimate of a primary unit total may be used more than once:

$$(7)\quad \hat{Y} = (1/n)\sum^{n} \hat{Y}_i/p_i$$

$$(8)\quad V(\hat{Y}) = (1/n)\sum^{N} p_i(Y_i/p_i - Y)^2 + (1/n)\sum^{N} \sigma_i^2/p_i$$

$$(9)\quad \hat{V}(\hat{Y}) = [1/n(n-1)]\sum^{n} (\hat{Y}_i/p_i - \hat{Y})^2.$$

Equations 7–9 are unbiased when one uses unbiased estimators at the second stage of sampling; if electrofishing is used at the second stage, equations 7 and 9 will be approximate.

PPS with replacement designs will perform best when Y_i are proportional to M_i. Then, $p_i = M_i/M_0 = Y_i/Y$, and the expected value of $Y_i/p_i = Y$ for all i; hence, the first term in equation 8 becomes zero. However, the possibility that the same primary unit may be included more than once in a sample means that PPS with replacement selection of primary units will usually be less efficient than PPS without replacement selection. When the sampling universe is large and the number of selected primary units is small, efficiency will be comparable with PPS without replacement designs. Chief virtues of PPS with replacement are (1) computation of selection probabilities is simple because the p_i are independent of sample size and (2) the sample-based estimator of variance (equation 9) does not require estimates of second stage variance.

Design D: PPS — Sampling without replacement

For this design, primary units are selected without replacement with probabilities proportional to their sizes according to one of many possible selection methods (see Hanif and Brewer 1980 for a review of 50 published unequal probability selection methods). Let π_i = probability that the ith primary unit will be in a sample of size n, and let π_{ij} = probability that units i and j will be in the sample ($i \neq j$). Assuming that unbiased estimators are used at the second stage of sampling, Raj (1968, p. 118–119) proved that unbiased two-stage estimators are

$$(10)\quad \hat{Y} = \sum_{i}^{n} \hat{Y}_i/\pi_i$$

$$(11)\quad V(\hat{Y}) = \sum_{i}^{N-1} \sum_{j>i}^{N} (\pi_i\pi_j - \pi_{ij})(Y_i/\pi_i - Y_j/\pi_j)^2 + \sum_{i}^{N} \sigma_i^2/\pi_i$$

$$(12)\quad \hat{V}(\hat{Y}) = \sum_{i}^{n-1} \sum_{j>i}^{n} \frac{(\pi_i\pi_j - \pi_{ij})}{\pi_{ij}} (\hat{Y}_i/\pi_i - \hat{Y}_j/\pi_j)^2 + \sum_{i}^{n} \hat{\sigma}_i^2/\pi_i$$

with the restrictions that all $\pi_{ij} > 0$ and that

$$\sum_{i}^{N} \pi_i = n \quad \text{and} \quad \sum_{i}^{N-1} \sum_{j>i}^{N} \pi_{ij} = n(n-1)/2.$$

Summations are over all possible distinct pairs of primary units in the sampling universe (equation 11) or in a particular sample of size n (equation 12). When electrofishing is used at the second stage of sampling, equations 10 and 12 are approximate.

Because primary units are selected without replacement, the efficiency of this design will almost always exceed that of a PPS with replacement design applied to the same sampling universe. This design will also perform best when Y_i are proportional to M_i. If the selection method ensures that π_i are proportional to M_i (not all methods will ensure this (see Appendix B)), then $Y_i/\pi_i = Y_j/\pi_j$ for all $i, j\ (i \neq j)$ and the first term in equation 11 becomes zero. For sample sizes exceeding 2, computation of the π_{ij} is not a simple task and requires use of a computer. Appendix B presents details of two PPS without replacement selection methods, whose performances are contrasted in the following section, and of formal properties of equations 11 and 12.

Performance of Alternative Designs

Relative performances of the four alternative designs depend primarily on the following conditions: (1) the number of primary units that are selected, n, and the size of the sampling universe, N, (2) the degree to which primary unit totals are proportional to primary unit sizes, measured roughly by the correlation between Y_i and M_i, (3) the range of sizes of primary units in the sampling universe, and (4) survey costs associated with electrofishing within selected primary units, as opposed to those costs that are independent of the sizes of those primary units that are selected. No single design will perform best under all possible conditions. Design A (SRS) may perform best when primary unit totals are poorly correlated with primary unit sizes, whereas design D (PPS without replacement) may perform best when primary unit totals are highly correlated with primary unit sizes. Comparison of the relative performances of alternative designs therefore requires their application to specific sampling universes; results depend on the characteristics of those sampling universes used as bases for comparison.

Construction of sampling universes

Sampling universes formally consist of unique sets of primary units with sizes and totals known exactly and are usually of two types: natural or artificial. Natural sampling universes have a real-life existence, whereas artificial sampling universes are imaginary and constructed. It is impossible to satisfy the formal requirements for a natural sampling universe in this stream survey context because (1) numbers of fish in primary units are always estimated rather than enumerated, (2) only a small fraction of primary units within a stream is ever sampled, and (3) selected primary units are almost always of equal sizes. However, one may arrive at a compromise between a natural and an artificial sampling universe by treating available estimates of primary unit totals and sizes as if they fulfilled the requirements of a natural sampling universe.

TABLE 1. Pool sizes (m^2) and estimated population sizes for pools sampled during 1981 and 1982 in Knowles Creek, Oregon, by the U.S. Forest Service.

1981		1982	
Pool size	Estimated population	Pool size	Estimated population
6686	2875	2641	5547
4757	1142	649	779
520	52	340	68
302	175	255	153
219	129	210	42
186	39	180	144
179	159	152	76
108	130	145	217
79.4	127	70.0	70
44.2	42	46.4	51
31.3	47	40.0	28
16.0	16	32.7	49
12.0	12	22.0	22
9.60	23	6.96	16
9.44	17	4.29	9

Estimated population sizes ($\hat{Y}_i$) of yearling coho salmon (*Oncorhynchus kisutch*) and surface areas (M_i) of entire pools sampled in a small Oregon coastal stream in each of two years (Table 1) were used to construct three pairs of such compromise sampling universes. For each pair of constructed sampling universes (1981 and 1982), the total number of sampled primary units (pools) was treated as the total size (N) of a small sampling universe, and estimated primary unit totals and sizes were treated as if they were exactly known totals and sizes. Constructed sampling universes were deliberately designed to differ in two principal respects, (1) the correlation between Y_i and M_i and (2) the range of Y_i and M_i, in order to present a useful contrast of the relative performances of alternative designs under differing conditions. Because these three pairs of constructed sampling universes were all small ($N \leq 15$), an additional, large ($N = 50$) artificial sampling universe was also constructed, based in part on Table 1. Primary unit totals and sizes for this large sampling universe are presented in Table 2.

Calculation of net relative efficiencies

The most useful single measure of the performance of alternative designs is net relative efficiency. Letting $V(\hat{Y})$ denote sampling variance and C denote total costs, then, for a fixed sampling fraction, n/N, the net relative efficiency of design B compared with design A is (Jessen 1978, p. 80, 112)

$$\text{NRE(B/A)} = V_A(\hat{Y})C_A/V_B(\hat{Y})C_B.$$

Net relative efficiency balances the relative efficiency of design B compared to design A ($\text{RE(B/A)} = V_A(\hat{Y})/V_B(\hat{Y})$) against the relative cost of design B compared with design A ($\text{RC(B/A)} = C_B/C_A$). For example, if design B is twice as precise as design A ($\text{RE(B/A)} = 2$), but total costs for design B are three times that for design A ($\text{RC(B/A)} = 3$), then the net relative efficiency of design B compared with design A is 2/3. Net relative efficiency is less than 1 because improvements in precision are more than

TABLE 2. Artificial sampling universe giving pool sizes and population sizes for all pools (constructed from those data presented in Table 1).

Pool no.	Pool size	Population
1	649	779
2	520	52
3	430	377
4	340	68
5	321	144
6	302	175
7	279	100
8	255	153
9	237	212
10	219	129
11	215	120
12	210	42
13	198	133
14	186	39
15	180	137
16	180	144
17	179	159
18	166	76
19	152	76
20	149	46
21	145	217
22	127	57
23	108	130
24	93.7	86
25	79.4	127
26	74.7	46
27	70.0	70
28	58.2	43
29	46.4	51
30	45.3	48
31	44.2	42
32	42.1	34
33	40.0	28
34	36.4	22
35	32.7	49
36	32.0	12
37	31.3	47
38	26.7	18
39	22.0	22
40	19.0	14
41	16.0	16
42	14.0	21
43	12.0	12
44	10.8	15
45	9.60	23
46	9.52	17
47	9.44	17
48	8.20	11
49	6.96	16
50	4.29	9

offset by increases in cost for design B: design B is less cost-effective than design A.

Calculation of net relative efficiency therefore requires calculation of (a) sampling variance and (b) total survey costs. In the example results that follow, equations 2, 5, 8, and 11 were used to calculate sampling variances for designs A, B, C, and D, respectively. For the PPS without replacement design (D), sampling variances were calculated for each of two different selection methods. For one of these selection methods, the magnitude of necessary computations limited maximum sample size to six for the small sampling universes and to three for the large sampling universe (see Appendix B). Calculations of second stage variances (σ_i^2) assumed use of a removal method estimator based on two passes of equal effort with electrofishing capture probability, q, set equal to 0.5 (see Appendix A; and Seber 1982, p. 318) and assumed to be independent of the sizes of the primary units. The possibility that q may be inversely related to primary unit size is also considered in Appendix A.

Based on conversations with biologists responsible for collecting those data presented in Table 1, relative costs for alternative designs were based on an assumption that when SRS was used to select primary units, half of survey costs were attributed to actual time spent electrofishing. Remaining costs, for housing and per diem for personnel, for time spent travelling to and from the study stream and between selected primary units, and for time spent setting up and taking down block nets prior to and after electrofishing, were assumed to be independent of the sizes of selected primary units. The time and cost spent to electrofish primary unit i was assumed to be proportional to M_i^b. For $b = 1$, time spent electrofishing primary unit i is directly proportional to actual primary unit size. For $b > 1$, larger units would require relatively more time than would be indicated by their actual sizes, M_i. Because in most situations b probably exceeds 1, b was set equal to either 1 or 1.5 for comparative purposes. For $b = 1.5$, M_i^b gives the *effective* size of primary unit i with respect to necessary electrofishing time. Thus, for example, the effective sizes of two primary units of actual sizes 100 and 1000 would be 1000 and 31 623; the ratio of actual sizes is 1:10, but that of effective sizes is about 1:32. Because M_i have been measured in terms of square metres, $M_i^{1.5}$ is a rough proxy for the volume of water that must be electrofished in primary unit i. Details of cost calculations are presented in Appendix C.

Because PPS designs (C and D) assign higher selection probabilities to larger primary units, the expected total sizes of n selected primary units for the PPS designs will always exceed those for the two SRS designs (A and B). Thus, for net relative efficiency of PPS designs compared with SRS designs to exceed 1, it is not sufficient that relative efficiency exceed 1; improvements in precision must more than compensate for increases in expected total costs for these designs. Net relative efficiencies (with respect to design A) were calculated for both actual and effective primary unit sizes for all designs and for both PPS without replacement selection methods, with one exception. For the large sampling universe, net relative efficiencies were calculated only for actual primary unit sizes ($b = 1$).

Results

For brevity in presentation of the relative performances of the four alternative two-stage sampling designs, the following notation has been adopted: (1) $V_A(\hat{Y})$, $V_B(\hat{Y})$, $V_C(\hat{Y})$, and $V_D(\hat{Y})$ denote sampling variances for design A (SRS), design B (SRS/ratio estimation), design C (PPS with replacement), and design D (PPS without replacement); (2) RC(B), RC(C), and RC(D) denote relative costs for designs B, C, and D (with respect to design A); and (3) NRE(B), NRE(C), and NRE(D) denote net relative efficiencies of designs B, C, and D (with respect to design A).

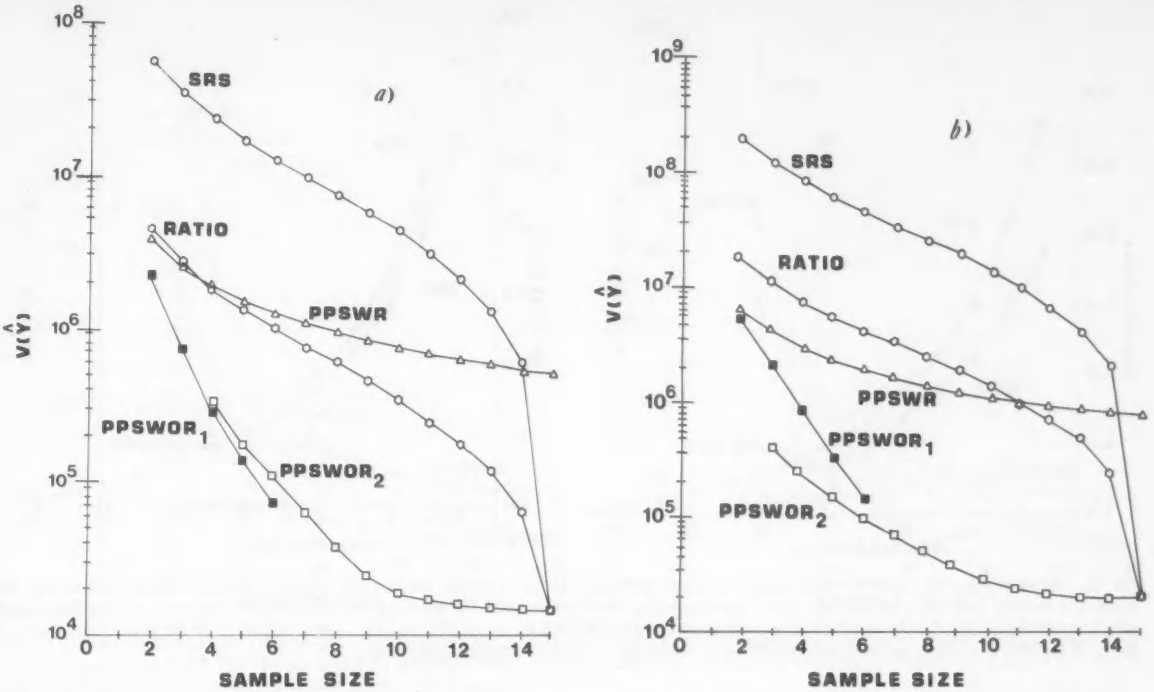

FIG. 1. Sampling variances, $V(\hat{Y})$, for SRS (SRS), SRS/ratio estimation (RATIO), PPS with replacement (PPSWR), and PPS without replacement (PPSWOR) designs plotted against sample size for example 1 in (a) 1981 and (b) 1982. Subscripts on PPSWOR indicate selection methods 1 and 2. Sample sizes for which more than one $\pi_{ij} = 0$ for the second selection method are not plotted. For the second selection method: 1981: $\pi_{10,11} = 0$ ($n = 10$), $\pi_{12,13} = 0$ ($n = 12$); 1982: $\pi_{3,4} = 0$ ($n = 3$), $\pi_{9,10} = 0$ ($n = 9$), $\pi_{13,14} = 0$ ($n = 13$), $\pi_{14,15} = 0$ ($n = 14$).

Example 1

When all data in Table 1 are used to construct two sampling universes, then $N = 15$ (for each) and the correlations between Y_i and M_i are 0.962 and 0.988 for 1981 and 1982. These strong correlations and the extreme ranges in primary unit sizes, 9.44–6686 and 4.29–2641 m^2, a priori suggest that designs B, C, and D should outperform design A.

Figure 1 shows that, for both 1981 and 1982, $V_A(\hat{Y})$ exceeds that for all other designs for $n < N$. $V_B(\hat{Y})$ is considerably less than $V_A(\hat{Y})$ and, for large n relative to N, $V_B(\hat{Y}) < V_C(\hat{Y})$. Design D (for both selection methods) is most precise for $n \geq 2$, and sampling variance declines most rapidly with increasing sample size for this design.

The penalty paid for the improved precision of the PPS designs (C and D) is a substantial increase in relative costs (Fig. 2). However, relative costs for the PPS without replacement design decline rapidly with increasing sample size. As a result of the high and constant relative cost of design C (see Appendix C), NRE(B) > NRE(C) for nearly all sample sizes for both years and for both actual and effective sizes of primary units. NRE(C) declines linearly with increasing sample size and, for $n \approx N$, is less than 1. For $n > 2$, NRE(D) exceeds that of any other design for both years and for both actual and effective primary unit sizes. Further, NRE(D) increases with increasing sample size until more than half of the primary units have been sampled; for all other designs, net relative efficiencies decline with increasing sample size (Fig. 3). Relative performances of the alternative designs are very similar for both 1981 and 1982, as is the case for all other examples, suggesting that relative performances may be fairly stable across years. For these two sampling universes, then, Design D (PPS without replacement) would be the clear design of choice (among the four alternatives) for $n > 2$. For $n = 2$, designs B and D have similar net relative efficiencies. (However, design B is not recommended for small n; see Discussion.)

Example 2

If the three largest primary units are removed from data sets in Table 1 for both years, then $N = 12$, the correlations between Y_i and M_i are reduced to 0.794 and 0.694, and the ranges of primary unit sizes decrease to about two rather than three orders of magnitude. $V_B(\hat{Y})$ is again less than $V_A(\hat{Y})$ for all $n < N$, but $V_C(\hat{Y})$ always exceeds $V_B(\hat{Y})$ for 1981 and does so for $n > 3$ in 1982. $V_D(\hat{Y})$ is smallest for all sample sizes (Fig. 4).

Because the range in sizes of primary units has been reduced, the relative costs for the PPS designs are much lower than for example 1 (Fig. 5). NRE(C) again declines linearly with increasing sample size and is less than 1 for $n > 3$ in most cases. NRE(B) exceeds 1 for all $n < N$ for both years and for both actual and effective primary unit sizes. NRE(D) exceeds NRE(B) for all sample sizes exceeding 4. For $n \leq 4$, either design B or design D has greatest net relative efficiency (Fig. 6). (However, design B is not recommended for small n; see Discussion.)

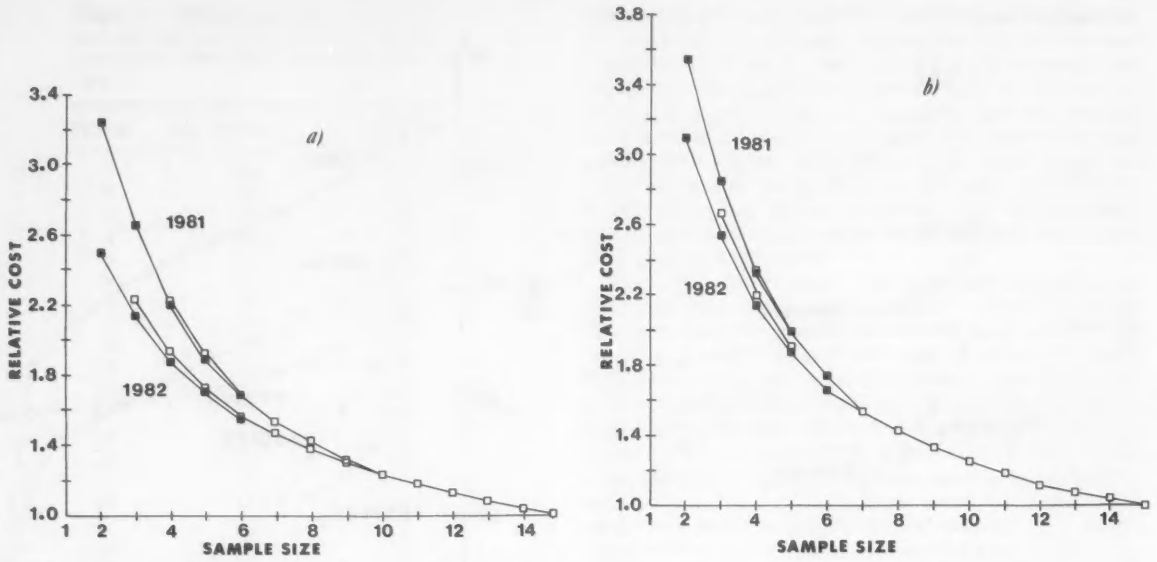

FIG. 2. Relative costs for the PPSWOR design, selection methods 1 (solid symbols) and 2 (open symbols), plotted against sample size for example 1 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Sample sizes for which one or more $\pi_{ij} = 0$ for the second selection method are listed in the caption to Fig. 1. Relative costs for PPSWR design in 1981 and 1982 were 3.44 and 3.03 for actual sizes and 4.24 and 5.20 for effective sizes. See text for explanation of actual and effective sizes of primary units.

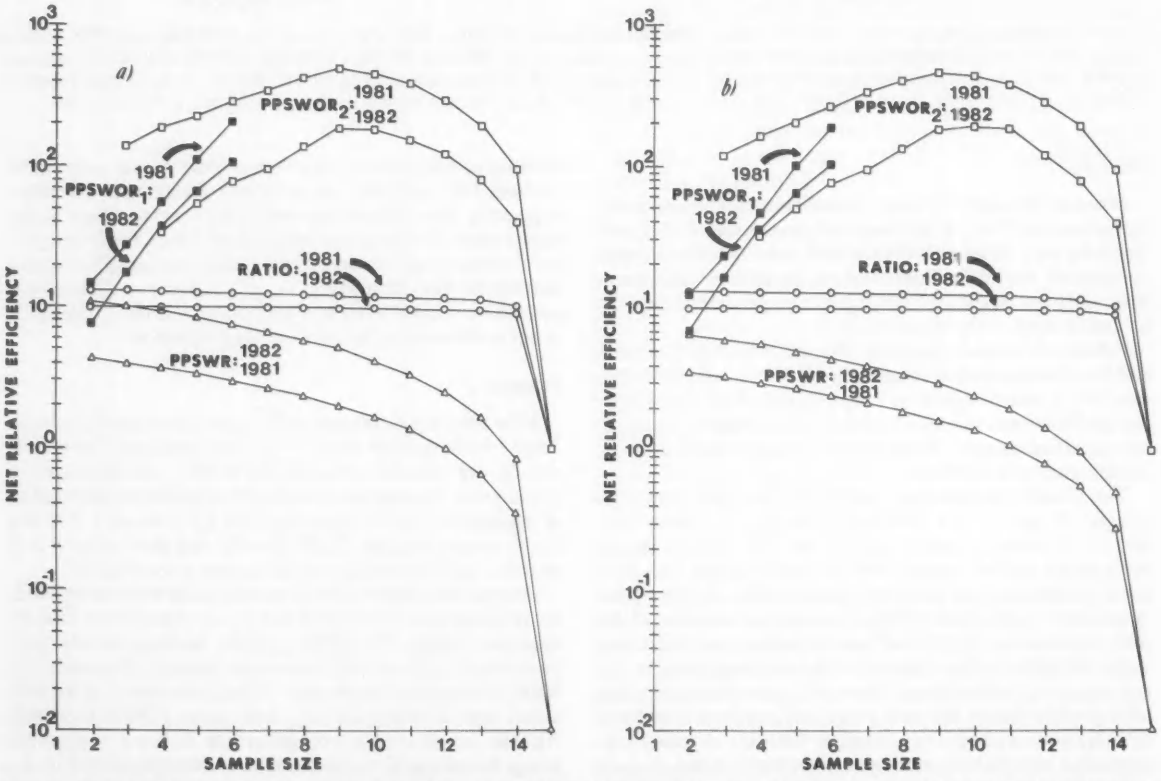

FIG. 3. Net relative efficiencies for RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 1 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Subscripts on PPSWOR indicate selection methods 1 and 2. Sample sizes for which one or more $\pi_{ij} = 0$ for the second selection method are listed in the caption to Fig. 1.

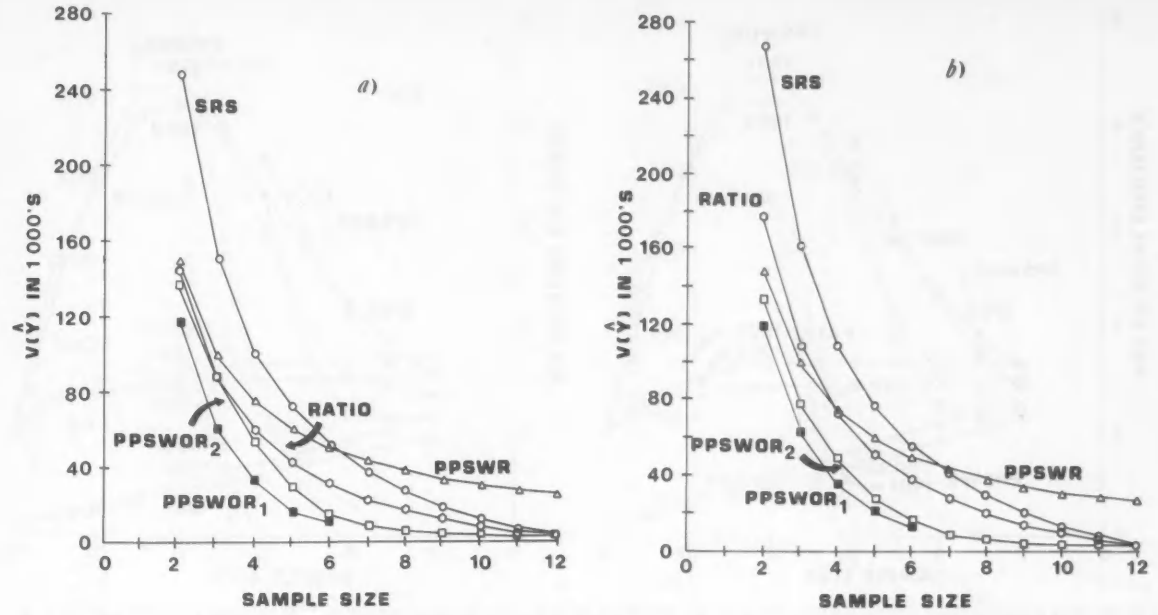

FIG. 4. Sampling variances, $V(\hat{Y})$, for SRS, RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 2 in (a) 1981 and (b) 1982. Subscripts on PPSWOR indicate selection methods 1 and 2. For the second selection method: 1981: $\pi_{6,7} = 0$ ($n = 6$), $\pi_{10,11} = 0$ ($n = 10$), $\pi_{11,12} = 0$ ($n = 11$); 1982: $\pi_{7,8} = 0$ ($n = 7$), $\pi_{9,10} = 0$ ($n = 9$).

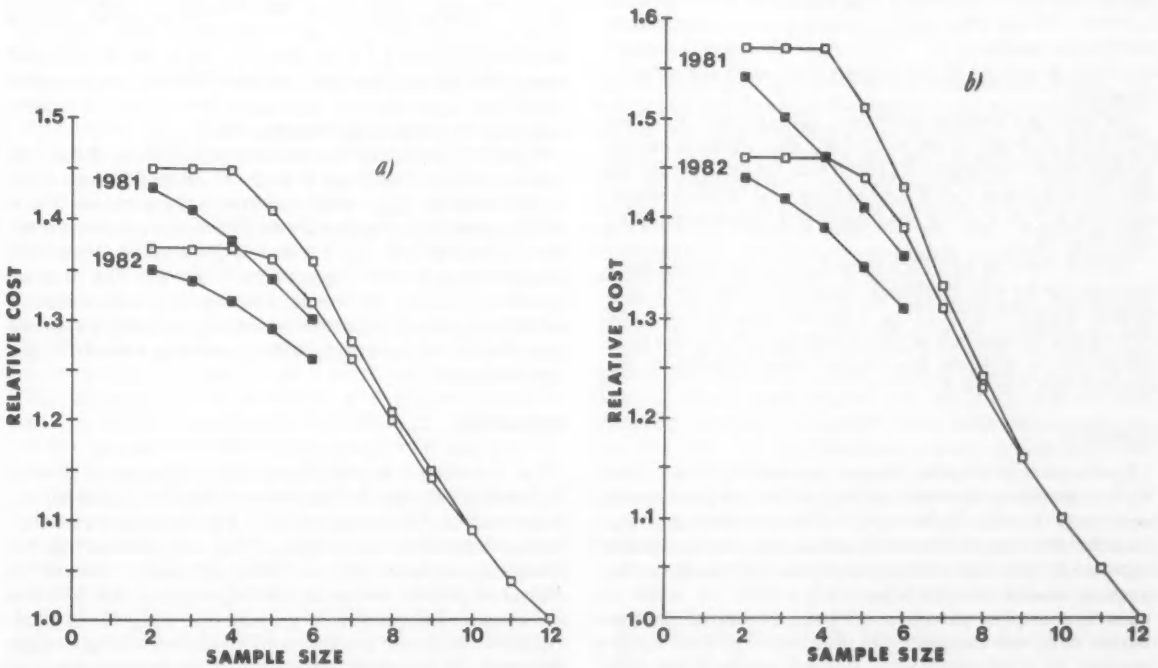

FIG. 5. Relative costs for the PPSWOR design, selection methods 1 (solid symbols) and 2 (open symbols), plotted against sample size for example 2 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Sample sizes for which one $\pi_{ij} = 0$ are listed in the caption to Fig. 4. Relative costs for PPSWR design in 1981 and 1982 were 1.45 and 1.37 for actual sizes and 1.74 and 1.59 for effective sizes.

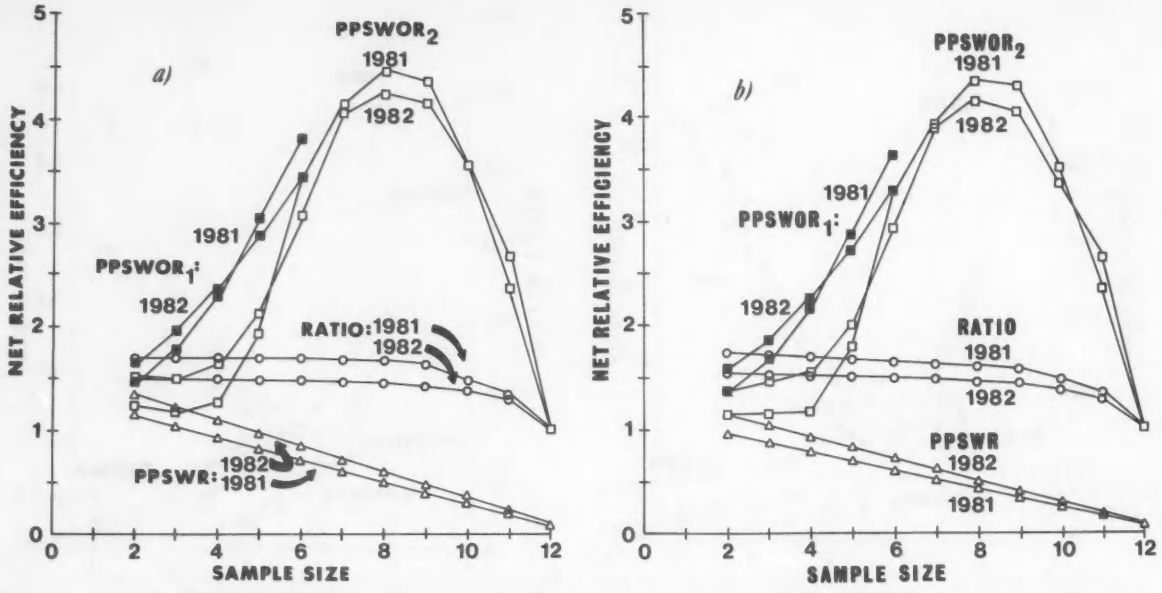

FIG. 6. Net relative efficiencies for RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 2 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Subscripts on PPSWOR indicate selection methods 1 and 2. Sample sizes for which one $\pi_{ij} = 0$ for the second selection method are listed in the caption to Fig. 4.

Example 3

If the two smallest and two largest primary units are removed from data sets for each year presented in Table 1, then $N = 11$ and the correlations between Y_i and M_i are reduced to 0.310 and 0.398 for 1981 and 1982; these correlations would not be judged statistically significant ($p > 0.05$). Also, the range in primary unit sizes is reduced to just slightly more than one order of magnitude.

For these two sampling universes, $V_B(\hat{Y})$ exceeds $V_A(\hat{Y})$ for all sample sizes and all cases; for 1981 the contrast is strong. $V_C(\hat{Y})$ is slightly less than $V_B(\hat{Y})$ for $n \leq 3$, but for larger sample sizes $V_C(\hat{Y})$ exceeds $V_B(\hat{Y})$; $V_C(\hat{Y})$ exceeds $V_A(\hat{Y})$ for all sample sizes. For $n > 2$, $V_D(\hat{Y})$ is less than for any other design for all cases (Fig. 7).

Relative costs for the PPS without replacement design again decrease rapidly with increasing sample size, and for $n > 5$, NRE(D) always exceeds 1 (Fig. 8 and 9). Thus, for $n > 5$, the PPS without replacement design would again be the design of choice. For $n \leq 4$, the best choice would probably be the simplest: SRS (design A).

Example 4

For the artificial sampling universe presented in Table 2, $N = 50$, the correlation between Y_i and M_i is 0.760, and primary unit sizes range from 4.29 to 649 m^2. This universe thus has characteristics that are intermediate between examples 1 and 2 (in terms of correlation and range of primary unit sizes), but the sampling universe is much larger.

For this sampling universe, $V_A(\hat{Y})$ exceeds that of all other designs with one exception: for $n > 40$, $V_A(\hat{Y}) < V_C(\hat{Y})$. However, for small sample sizes, design C performs very well: for $n < 27$, $V_C(\hat{Y}) < V_B(\hat{Y})$; and for $n \leq 3$, $V_C(\hat{Y}) \approx V_D(\hat{Y})$. For sample sizes exceeding 3, $V_D(\hat{Y})$ is less than that for any other design (Fig. 10).

For $n < 8$, NRE(C) exceeds that for design B, and for $n \leq 3$, both PPS designs have comparable net relative efficiencies. NRE(D) increases dramatically with increasing sample size from $n = 2$ through at least $n = 28$ (Fig. 11). With the exception of very small sample sizes, then, design D (PPS without replacement) would again be the design of choice. For very small sample sizes ($n \leq 3$), design C might be the preferred choice due to the simplicity of the PPS with replacement calculations and the fact that equation 9 does not require estimates of second stage variances ($\hat{\sigma}_i^2$).

Figure 12 shows that the striking performance of the PPS without replacement design is achieved entirely through rapid reduction in first stage variance as sample size increases. For $n \leq 30$, second stage variance for the PPS design exceeds that for the SRS design (A), and for $n \geq 27$, second stage variance actually exceeds first stage variance for the PPS without replacement design. In contrast, first stage variance is at least an order of magnitude larger than second stage variance for $n < 40$ and is always large compared with second stage variance for the SRS design (A).

Discussion

The preceding examples illustrate that choice among alternative sampling designs for small streams depends sensitively on characteristics of sampling universes and on sampling fractions. Choice depends far less sensitively on electrofishing capture probability, q (even if q is more realistically allowed to depend on primary unit sizes; see Appendix A). For $q = 0.5$ and assumed independent of primary unit size, second stage variance was almost always small compared with first stage variance. The exception to this general result was for design D (PPS without replacement). For large sampling fractions (>50%), second stage variance exceeded first stage variance for this design (Fig. 12). However, one would rarely expect to

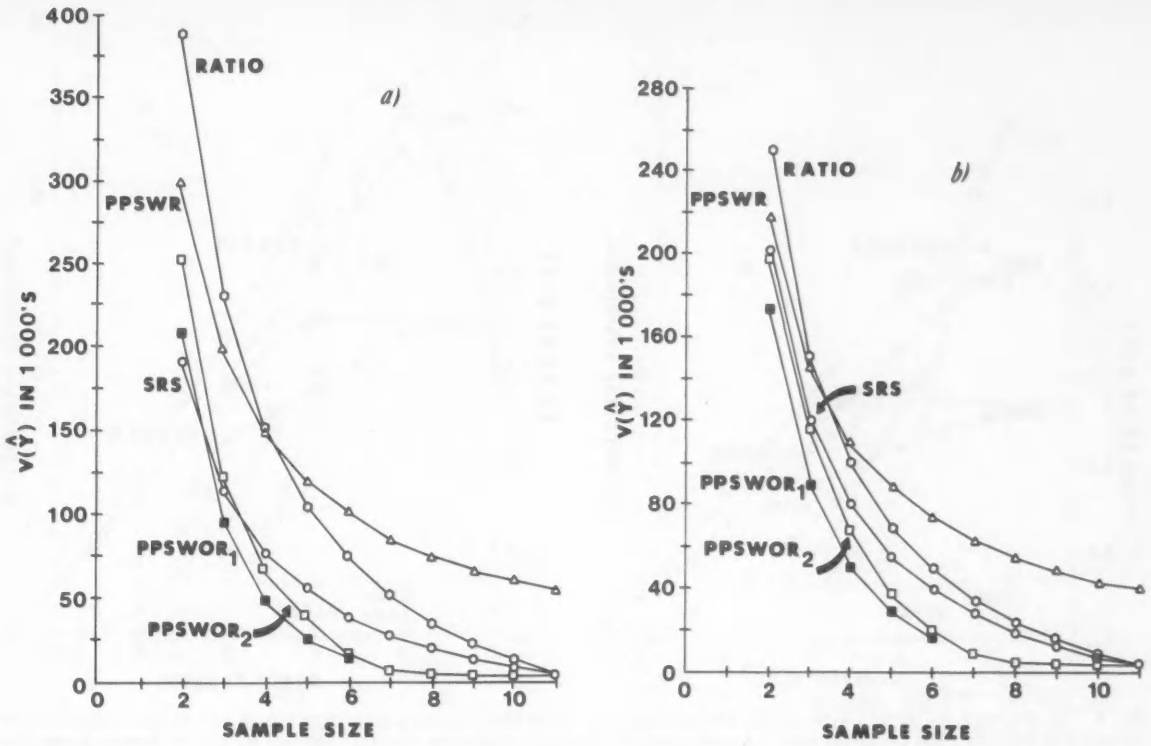

FIG. 7. Sampling variances, $V(\hat{Y})$, for SRS, RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 3 in (a) 1981 and (b) 1982. Subscripts on PPSWOR indicate selection methods 1 and 2. For the second selection method: 1981: $\pi_{8,9} = 0$ ($n = 8$), $\pi_{10,11} = 0$ ($n = 10$); 1982: $\pi_{7,8} = 0$ ($n = 7$).

achieve such a large first stage sampling fraction in fisheries work.

Interestingly, far more attention has been paid to electrofishing capture probability (and hence, second stage variance) in small streams than to reduction in first stage variance through choice of two-stage sampling design (e.g. Bohlin 1982). Preoccupation with electrofishing capture probability has no doubt resulted from the convention of selecting primary units of equal sizes. When stream sections (primary units) are of equal sizes, there are no substantive alternatives to SRS or systematic selection of primary units and there are usually no obvious means whereby an auxiliary variable, such as primary unit size, can be used to increase the precision of resulting estimates. When primary units are allowed to vary in size, alternative two-stage sampling designs can substantially reduce first stage variance and substantially increase precision of estimation of the total number of fish in small streams.

Although results presented in this paper assumed that a removal method estimator, based on electrofishing, was used at the second stage of sampling, presented formulas and designs would remain valid and unaltered were some other population estimator, e.g. a mark–recapture estimator, used at the second stage of sampling. Ideally, estimators used at the second stage of sampling should be unbiased. Given unbiased second stage estimators, the formulas presented in this paper are unquestionably valid and, with the exception of design B (SRS/ratio estimation), formally unbiased. However, the author is not aware of any existing population estimators that are unbiased and also applicable to the small stream context. Of course, the use of an explosive (primacord) could result in direct enumeration of primary unit totals; second stage error is entirely eliminated by this method. When sample sizes are very small, such an approach may have merit. However, if one restricts oneself to the usual stream population estimators, mark–recapture and removal method, one must recognize that estimates of primary units are biased; therefore, presented formulas must be regarded as approximations.

When electrofishing is used at the second stage and capture probability exceeds 0.5, then the relative magnitude of first stage variance compared with second stage variance may present an even more dramatic contrast than that indicated by Fig. 12. Thus, when primary units are of unequal size, the relative performances of alternative two-stage sampling designs are diluted very little in the stream survey context from what their relative performances would have been in a one-stage setting. It is worth noting that this would not usually be the case in more typical commercial fishery applications of alternative two-stage sampling designs. When there are many discrete subunits at the second stage and second stage sampling fractions are small, the relative performances of alternative two-stage sampling designs may be very similar; second stage variance may be large compared with first stage variance (see Cochran 1977, p. 310).

In the stream survey context, choice among alternative sampling designs rests almost entirely on the characteristics of the sampling universe (the correlation between Y_i and M_i and

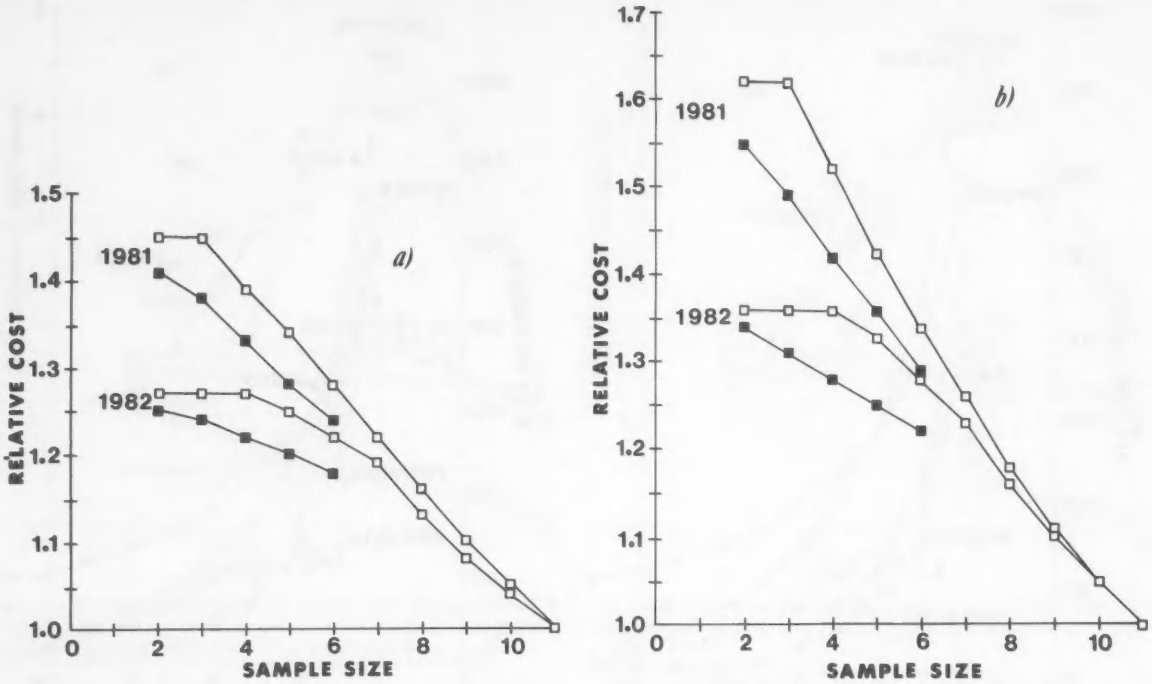

FIG. 8. Relative costs for the PPSWOR design, selection methods 1 (solid symbols) and 2 (open symbols), plotted against sample size for example 3 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Sample sizes for which one $\pi_{ij} = 0$ for the second selection method are listed in the caption to Fig. 7. Relative costs for PPSWR design in 1981 and 1982 were 1.45 and 1.27 for actual sizes and 1.88 and 1.49 for effective sizes.

the size of the sampling universe) and on sampling fraction. Examples provided calculations of net relative efficiencies of four alternative designs applied to three sets of small ($N \leq 15$) sampling universes, with a broad contrast of correlations between Y_i and M_i, and to one large ($N = 50$) sampling universe for which the correlation between Y_i and M_i was large (0.760) but realistic. Based on these examples, it is possible to make some fairly general, if crude, recommendations for the use of these four alternative designs when primary units are of unequal sizes.

For small sampling universes (e.g. $N < 20$) neither design B nor design C can be recommended regardless of the correlation between Y_i and M_i or of sampling fraction. PPS with replacement will perform poorly relative to PPS without replacement in small sampling universes, and the use of SRS/ratio estimation cannot be recommended because of possible serious underestimation in sample-based estimates of variance when sample sizes are small ($n < 12$; Cochran 1977, p. 162–164). One is thus left to choose between the SRS design (A) and the PPS without replacement design (D). When the correlation between Y_i and M_i exceeds about 0.5, PPS without replacement appears to be the design of choice, regardless of sample size. For small sample sizes (e.g. $n \leq 5$) the first PPS without replacement selection method can be used; for larger sample sizes the first selection method cannot be used, but the second selection method (Chao 1982) can be used (see Appendix B). For correlations between 0.3 and 0.5, choice of design appears to depend on sample size; for small sample sizes the SRS design is probably the design of choice, whereas for larger samples Chao's method of selecting PPS without replacement samples is recommended. When the correlation between Y_i and M_i is less than 0.3, the SRS design is recommended; the correlation is so low that one cannot take advantage of the potential benefits of PPS without replacement selection.

For large sampling universes ($N \approx 50$) choices are less clear-cut. When the correlation between Y_i and M_i exceeds 0.5, then design C (PPS with replacement) is recommended for small samples; the performances of PPS with and without replacement designs are so similar for small sample sizes that one cannot justify the additional computational complexities of the without replacement design. For larger sample sizes, Chao's method of selecting PPS without replacement samples is recommended and will outperform the with replacement design. For correlations less than 0.3, the SRS design is again the design of choice, and for correlations between 0.3 and 0.5 recommendations follow those for small sampling universes. Finally, for very large sampling universes (e.g. $N > 100$) with correlations between Y_i and M_i exceeding 0.5, PPS with replacement is recommended for small samples (e.g. $n \leq 12$); for larger samples, design B (SRS/ratio estimation) is recommended. Formulas for the SRS/ratio estimation design become approximately valid for large sample sizes selected from large sampling universes, and the PPS without replacement design is simply too unwieldly to use.

The author makes the above recommendations with some reluctance for two reasons. First, they are based on a comparison of the relative performances of alternative designs as applied to a limited and specific set of sampling universes.

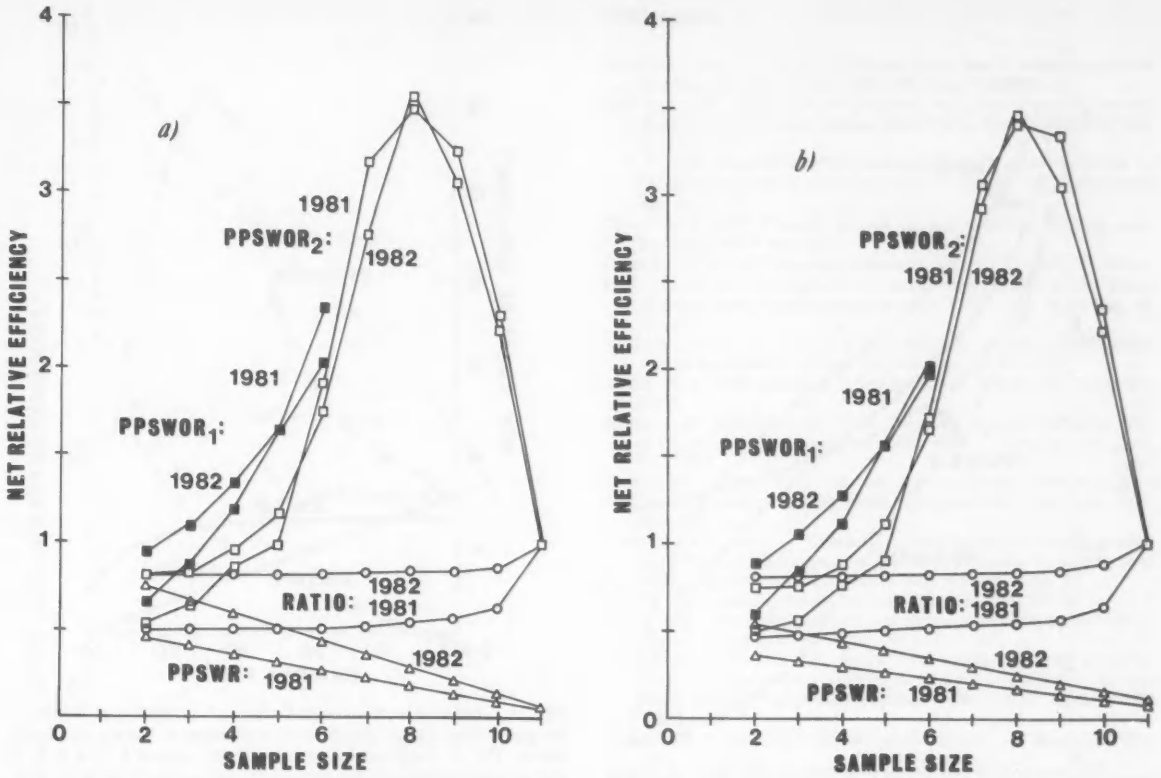

FIG. 9. Net relative efficiencies for RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 3 in 1981 and 1982. (a) Actual sizes of primary units; (b) effective sizes. Subscripts on PPSWOR indicate selection methods 1 and 2. Sample sizes for which one $\pi_{ij} = 0$ for the second selection method are listed in the caption to Fig. 7.

Second, they strongly support the use of PPS without replacement designs in many contexts when the correlation between Y_i and M_i exceeds about 0.3. It is only recently that methods such as Chao's (1982) have been developed and have allowed extension of the PPS without replacement selection method to large sample sizes drawn from large sampling universes. Both PPS without replacement methods that were used in this paper require large numbers of computations (for $n > 2$). These computations required use of a computer; in the case of Chao's method one must use a computer to select a particular sample. It is clear, however, that for $N \approx 50$ and $n > 5$ Chao's method performs well and is feasible in a practical fisheries context. The author constructed his own computer programs (in APL) for implementing both methods of PPS without replacement selection that were used in this paper and is unaware of any packaged programs for these selection methods, although they may indeed exist.

Because of the above considerations, it is probably worthwhile to stress the generally solid performance of the SRS/ratio estimation design for moderate and large sample sizes ($n > 12$) drawn from large sampling universes for which the correlation between Y_i and M_i exceeds 0.5. There are two significant advantages of this design. First, computations are simple, can be done on a hand calculator if desired, and are easily extended to any sample size and any size sampling universe. Second, because primary units are selected by SRS (as in design A), one can always compare the performances of this design and the SRS design. That is, having selected n primary units by SRS, one may always incorporate a measure of the sizes of selected primary units (using equation 6) and determine whether there would be any gain in precision over results from design A (using equation 3). There would be no additional cost imposed for this comparison if the sizes of selected primary units were to be measured anyway for other purposes. However, absent knowledge of the total size of all primary units (M_0), one could not use SRS/ratio estimation to estimate the total over all primary units (see equation 4).

The fact that SRS/ratio estimation and PPS designs require knowledge of the total size of all primary units may seriously compromise the validity of net relative efficiency calculations that have been presented in this paper. As they have been presented, the PPS designs require a map of a stream that specifies the sizes of all primary units. The SRS/ratio estimation design requires knowledge of the location of all units, the total size of all units (M_0), and the sizes of particular units that are selected in a sample. In contrast, because the SRS design (A) does not incorporate a measure of the sizes of selected units, this design requires only a map of the location of all primary units. Thus, the relative costs for the SRS/ratio estimation and PPS designs might be far greater than those calculated in this paper. However, it is likely that very simple measures of the sizes of primary units may provide excellent measures for the assign-

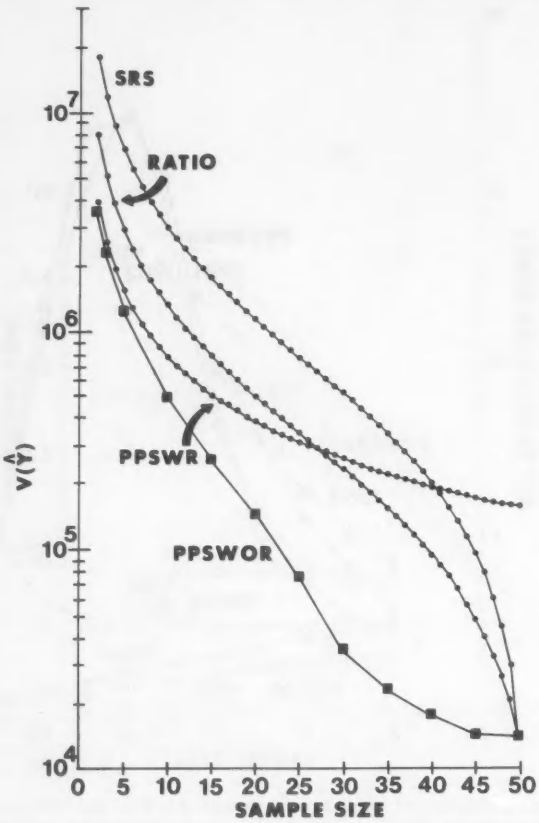

FIG. 10. Sampling variances, $V(\hat{Y})$, for SRS, RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 4. For the PPSWOR designs, $V(\hat{Y})$ is computed following selection method 1 for $n = 2, 3$ and for other sample sizes following selection method 2. No $\pi_{ij} = 0$ for any sample size for the PPSWOR design.

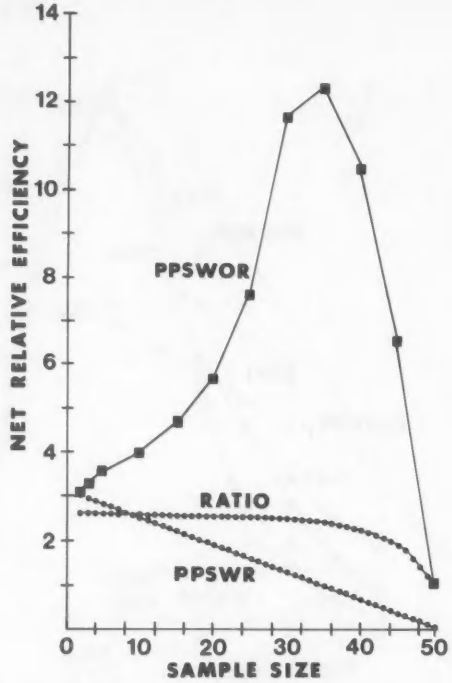

FIG. 11. Net relative efficiencies for RATIO, PPSWR, and PPSWOR designs plotted against sample size for example 4. For the PPSWOR design, $V(\hat{Y})$ is computed following selection method 1 for $n = 2, 3$ and for other sample sizes following selection method 2. No $\pi_{ij} = 0$ for any sample size for the PPSWOR design.

ment of PPS selection probabilities with little loss of efficiency. For example, if a particular reach of stream were of fairly uniform width, then the length of a pool could be a measure of size that would be highly correlated with pool area. This much simpler and less costly to obtain measure of primary unit sizes could then be used to assign PPS selection probabilities. The marginal cost of obtaining such simple measures of primary unit size would probably be small compared with the cost of locating all primary units; all primary units must be located for all designs. The author hopes to investigate this possibility in the future because of its obvious practical relevance for possible use of PPS designs.

For preliminary field research one usually has no existing data from which one could construct plausible sampling universes and conduct comparisons like those performed in this paper. Given such circumstances it is probably best to first sample primary units by SRS and then to compare the precisions of estimates based on designs A and B (assuming that sample sizes are large enough to warrant confidence in variance estimates from design B). If the sample correlation between Y_i and M_i is high, then future sampling might take advantage of this correlation by using one of the PPS designs following those general recommendations made previously. One should definitely not attempt immediate use of PPS designs when variation in primary unit sizes is small or when the correlation between Y_i and M_i is unknown or small. Preliminary field results can also give a great deal of insight into formation of strata within which alternative sampling designs could be used. Because habitat quality may vary markedly with location within a stream, the correlation between Y_i and M_i will probably be strongest within reaches of a stream that are most similar. Pools of the same size in a downstream, low-gradient region of a stream may hold very different numbers of fish than may pools of the same size in upstream, high-gradient regions. One might therefore choose to construct numerous small strata on the basis of general habitat quality and location within a stream, e.g. headwater pools for which stream gradient exceeds some specific amount, and to sample independently from each of these strata. It is very unlikely that there would be a strong correlation betwen Y_i and M_i over the entire length of any substantial stream. Only an intimate biological understanding of the stream to be sampled can lead to the most intelligent and effective construction of such strata; statistical advice alone is insufficient.

Finally, the author wishes to once more stress the importance of allowing primary unit sizes to vary according to natural habitat unit sizes. Regardless of the choice (or choices) that one makes among alternative two-stage sampling designs for small streams, one should minimize nonsampling errors that result from placement of block nets when primary units are essentially equivalent to natural habitat units. Also, one can learn far more

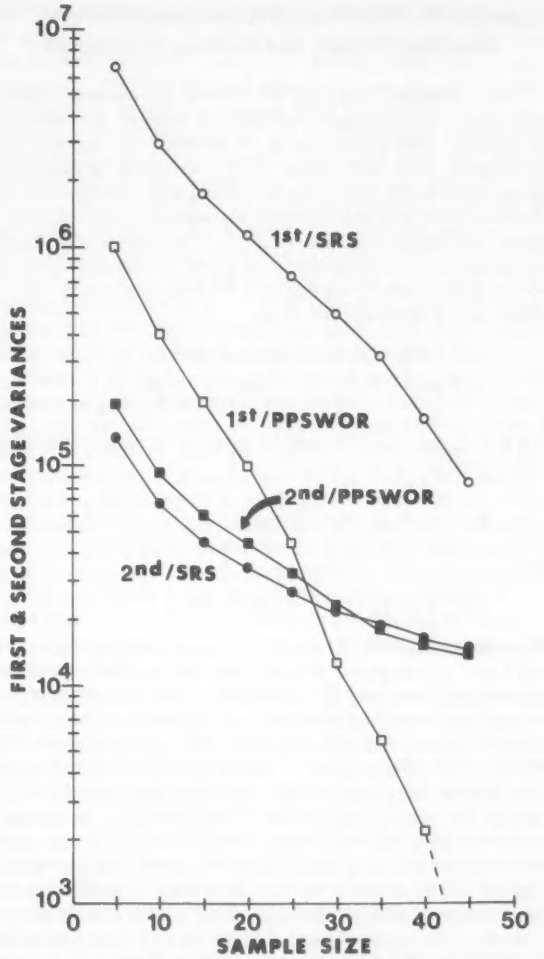

FIG. 12. First and second stage variances for SRS (1st/SRS and 2nd/SRS) and PPS without replacement (1st/PPSWOR and 2nd/PPSWOR) designs for example 4. All PPS calculations were based on selection method 2 and sample sizes are in increments of five.

about the relationships between fish abundance and distribution and habitat unit size and quality when one adopts the practice of allowing primary units to vary in size. After all, fish live in natural habitat units that we choose to call pools, runs, or riffles. We should sample them in their homes.

Acknowledgments

I extend sincere thanks to Fred Everest for his encouragement throughout the development of this paper, for permission to present the Knowles Creek data (without which a realistic appraisal of the relative performances of alternative sampling designs would have been impossible), and for his practical insights, which are reflected especially in Appendices A and C. I also extend thanks to A. R. Sen and D. S. Robson for their reviews of drafts of this paper; the discussion was substantially improved as a result. Support for publication was provided by the Humboldt State University Foundation and by the Pacific Northwest Forest and Range Experiment Station, U.S. Department of Agriculture.

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Appendix A. Calculation of Second Stage Variances, σ_i^2, for the Removal Method Estimator

Calculation of the σ_i^2 assumed that electrofishing was used to estimate a primary unit total based on two "passes" of equal effort. Prior to electrofishing, block nets are set to delimit primary unit boundaries. On the first pass, Y_i fish are available for capture, and of these, C_1 fish are captured by electrofishing and removed from the primary unit. On the second pass, $Y_i - C_1$ fish are available for capture and C_2 fish are captured. Then, if (a) $C_1 > C_2$ and (b) capture probability, q (the probability that a fish present at the time of a given pass will be captured) is assumed to be constant, one may estimate the primary unit total as (Seber 1982, Sect. 7.2)

$$(13)\quad \hat{Y}_i = C_1^2/(C_1 - C_2)$$

$$(14)\quad \sigma_i^2 = V(\hat{Y}_i) \approx Y_i(1 - q)^2(2 - q)q^{-3}.$$

$\hat{Y}_i$ is a maximum likelihood estimator and is therefore asymptotically unbiased. However, $\text{BIAS}(\hat{Y}_i) \approx (1 - q)(2 - q)q^{-3}$, which may be significant for small Y_i and small q. $V(\hat{Y}_i)$ is based on the delta method (Seber 1982, Sect. 1.3.3.) and is a large sample result.

For small Y_i (e.g. <50) and small q (<0.5), bias of equation 13 may be substantial relative to Y_i. For example, for $Y_i = 50$ and $q = 0.25$, then BIAS($\hat{Y}_i$)/Y_i is nearly 50%. In contrast, for large $q(q \geq 0.8)$, BIAS($\hat{Y}_i$) < 0.5 and squared bias is almost always insignificant compared with $V(\hat{Y}_i)$. These kinds of considerations have been recently examined in detail by Bohlin (1982). Peterson and Cederholm (1984) showed that the assumption that q is constant may not be valid for small streams, and Schnute (1983) developed alternative removal method estimators for situations when this assumption is violated.

In the example computations presented in this paper, q was set equal to 0.5, a fairly low figure, so as not to minimize the possible importance of second stage variance in the stream survey context. The author recognizes that if q were as low as 0.5, then many estimates of Y_i and σ_i^2 would be tenuous for Y_i < 100. In addition, q was assumed to be independent of primary unit size. However, the usual finding in small streams is that, instead, q is inversely related to primary unit size. One generally has large q (e.g. $\geq$0.8) for small (usually shallow) pools and small q (e.g. $\leq$0.5) for large (usually deep) pools. For the Knowles Creek data presented in Table 1, $0.8 \leq \hat{q} \leq 1$ for all pools < 140 m^2; for larger pools, $\hat{q}$ decreased with increasing pool size.

If q varies inversely with primary unit size, then σ_i^2 may dramatically increase with primary unit size when Y_i and M_i are highly correlated (see equation 14). However, this kind of dependence of q on primary unit size did not result in adverse impacts on relative performances of the PPS designs that are presented in this paper, even though these designs assigned higher selection probabilities to primary units of greater sizes and greater σ_i^2. In fact, when q was allowed to vary with primary unit size and to mimic results from the Knowles Creek data, numerical computations (not presented in this paper) showed that the relative performances of the PPS designs could actually be improved.

Lack of adverse impact on the relative performances of the PPS designs appears to be related to weightings for the second stage variance terms for the four alternative two-stage designs. Weightings for the σ_i^2 were N/n for SRS and ratio estimation (equations 2 and 6), $1/np_i$ for PPS with replacement (equation 8), and $1/\pi_i$ for PPS without replacement (equation 11). For small n relative to N, N/n is large and the two SRS designs give this same large weighting to all primary units without regard to primary unit size or σ_i^2. In contrast, for the larger primary units, for which the σ_i^2 would also be far larger, the PPS designs will have large p_i or π_i and the weightings for these larger units are often less than for the SRS designs. For the large sampling universe for which $N = 50$ and with $n = 2$, the SRS weightings are all 25, while both PPS weightings are about 5 for the largest primary unit. Of course, for the smaller primary units, weightings are reversed and the PPS weightings will be far larger than the SRS weightings. However, for these smaller units the primary unit total (Y_i) will be small and capture probability will be large ($q \geq 0.8$) so that the σ_i^2 for these smaller units are effectively negligible when compared with σ_i^2 for the larger primary units. As n approaches N, all SRS and PPS without replacement weightings are approximately the same and approach 1. Weightings will not stabilize with increasing sample size for the PPS with replacement design (because the p_i are independent of sample size), but this design is not recommended for large sample sizes (see Discussion).

Appendix B. PPS Without Replacement Designs: Selection Methods and Variance Estimators

Two methods of selecting PPS without replacement samples were used for this paper. For the first method, the first unit is selected with probability $p_i = M_i/M_0$. The second and subsequent units are drawn with conditional probabilities proportional to the sizes of the remaining units. Computation of these conditional selection probabilities requires only manipulation of the original p_i. Let $z_i, z_j, z_k, \ldots, z_n$ denote the conditional probabilities of selecting primary unit i on the first draw, j on the second given i drawn on the first, k on the third given i and j drawn on the first two, etc. Then:

$$z_i = p_i;\ z_j = p_j/(1 - p_i);\ z_k = p_k/(1 - p_i - p_j);\ \ldots;$$
$$z_n = p_n/(1 - p_i - p_j - p_k - \ldots - p_{n-1});$$
$$i \neq j \neq k \neq \ldots \neq n.$$

For $n = 2$, this selection method gives the explicit results (Raj 1968, p. 51):

$$\pi_i = p_i + \sum_{j \neq i}^{N} p_j p_i/(1 - p_j)$$

and

$$\pi_{ij} = p_i p_j/(1 - p_i) + p_j p_i/(1 - p_j).$$

Thus, the probability that a sample of size 2 contains the units i and j (π_{ij}) is computed as the sum of the products of (a) the probability of selecting the ith (or jth) unit on the first draw and (b) the conditional probability of selecting the jth (or ith) unit on the second draw given that the ith (or jth) unit was selected on the first draw. This amounts to summing the probabilities of the two ordered selections in which both primary units i and j appear. For $n > 2$, the probability of selecting any ordered sample of size n that contains the specified units $i, j, k, \ldots, n$ can be computed as $z_i z_j z_k \ldots z_n$. Then, the probability that unit i appears in the sample, π_i, is computed as the sum of the probabilities of all those ordered selections that contain unit i. Similarly, π_{ij} may be computed as the sum of the probabilities of all those ordered selections in which both the units i and j appear. Because calculation of the π_i and π_{ij} by this selection method requires construction of all possible ordered samples, it has been termed an "all possible samples" selection method (Hanif and Brewer 1980). Note that there are $N!/(N - n)!$ possible ordered samples.

Selection of primary units with probabilities proportional to the sizes of remaining units (method one) has several disadvantages: (1) the sheer magnitude of computations necessary to calculate the π_i and π_{ij} strictly limits practical sample size, (2) there is distortion of inclusion probabilities in that the π_i are not proportional to M_i, and (3) the method does not guarantee that all $\pi_{ij} < \pi_i \pi_j$ (see below). However, for $n = 2$, calculations are rapid and explicit, and for small n and N this selection method may prove to be practical and may perform well (see Results).

The second selection method used in this paper was recently proposed by Chao (1982) and is of an entirely different character. Chao's method avoids the need to construct all possible samples and ensures that π_i are proportional to M_i for most units. One first orders the primary units and then selects the first n units. Successive units from $n + 1$ through N are then

selected according to a probability scheme such that if the $n+i$th unit is selected, it replaces one of those units that was previously in the sample; sample size remains fixed. The π_i may be read directly from the last column of an easily constructed "normalized" probability matrix, and calculation of the π_{ij} follows explicit formulas (for any n) that readily lend themselves to computer algorithms. The advantages of Chao's method are (1) the number of computations necessary to calculate the π_i and π_{ij} is dramatically reduced as compared with all possible sample methods, thus allowing one to draw large samples from large sampling universes (e.g. $N \approx 50$), (2) for all $\pi_i < 1$, the π_i are strictly proportional to M_i, and (3) the method ensures that all $\pi_{ij} < \pi_i\pi_j$. However, Chao's method has the following disadvantages: (1) ordering of primary units will affect the π_{ij}, (2) the method does not guarantee that all $\pi_{ij} > 0$, and (3) certain π_i may equal 1: a unit may be selected with certainty. The author's experience has been that some π_{ij} may equal zero for very small ($n < 4$) or very large ($n \approx N$) sample sizes when primary units are ordered by decreasing size; for intermediate sample sizes usually all $\pi_{ij} > 0$. Thus, the two selection methods appear to complement one another. For very small samples drawn from moderate sized sampling universes (e.g. $N < 25$), it is practical to use the first selection method; for small sample sizes, Chao's method exhibits its disadvantages. For larger samples drawn from large sampling universes ($N \approx 50$), Chao's method will perform well whereas the first method will be impossible to apply due to the sheer magnitude of necessary computations (except for $n = 2$).

These formal details of the two PPS without replacement selection methods used in this paper are relevant for two reasons: (a) they serve to show that neither selection method can be used for all sample sizes and for all sampling universes; and (b) the formal properties described above may have serious consequences for the validity of using equations 11 and 12. First, equations 11 and 12 both require that all $\pi_{ij} > 0$; for some sample sizes, Chao's method would not meet this requirement. Second, although Sen (1954) proved that, for $n = 2$, the first selection method (or any other PPS without replacement selection method) will ensure that all $\pi_{ij} < \pi_i\pi_j$ so that equations 11 and 12 will always be positive, for $n > 2$ one has no such assurance. This means that if $\pi_{ij} > \pi_i\pi_j$ (for some i, j), certain pairs of primary units may actually make a negative contribution to variance (see equations 11 and 12). In fact, it is theoretically possible that equation 11 (and 12) may result in negative variance (estimates)!

For those example computations performed for this paper, it was always true that $\pi_{ij} < \pi_i\pi_j$ for the first selection method so that there were no possibilities of negative contributions to variance. For Chao's method of selection, primary units were ordered by decreasing sizes, and data points plotted on figures are such that no more than one $\pi_{ij} = 0$. Thus, on some figures there are no points plotted for Chao's selection method for very small n. Figure captions list those points for which a single $\pi_{ij} = 0$.

Appendix C. Calculation of Relative Costs

Let C denote total survey costs for sampling n primary units of unequal sizes that are selected by SRS, and assume that exactly half of these costs are attributable to time spent electrofishing the selected units. Then:

$$C = 0.5C + \gamma X_{SRS}$$

where γ = cost per unit of (actual or effective) primary unit size and X_{SRS} = expected total (actual or effective) size of n primary units selected by SRS. Normalize the total cost of sampling these n units by setting $C = 1$. Then for a specified n:

$$\gamma = 0.5/X_{SRS}.$$

Note that γ is a function of sample size, n. Relative costs for alternative sampling designs may then be calculated as $0.5 + \gamma X_{alt}$, where X_{alt} denotes the expected total (actual or effective) size of n primary units selected by some alternative method.

Expected total (actual or effective) sizes of n selected primary units for the four alternative designs are

$$nM_0/N\text{(actual) and } n\sum^{N} M_i^{1.5}/N\text{(effective)}$$

for SRS and SRS/ratio estimation,

$$n\sum^{N} M_i p_i\text{(actual) and } n\sum^{N} M_i^{1.5} p_i\text{(effective)}$$

for PPS with replacement, and

$$\sum^{N} M_i\pi_i\text{(actual) and } \sum^{N} M_i^{1.5}\pi_i\text{(effective)}$$

for PPS without replacement.

Recall that π_i is a function of sample size. Let X_{WR} and X_{WOR} denote the expected total (actual or effective) sizes for the PPS with and without replacement designs. Then the relative costs for these designs with respect to the SRS design(s) would be

$$\text{RC(PPSWR/SRS)} = 0.5 + \gamma X_{WR}$$

and

$$\text{RC(PPSWOR/SRS)} = 0.5 + \gamma X_{WOR}.$$

Because the π_i for the two PPS without replacement methods will differ, expected total (actual or effective) sizes of n selected units were computed for both of these selection methods.

Note that the expected total sizes of selected primary units are strictly proportional to sample size for both SRS designs and for PPS with replacement. This means that the relative cost of PPS with replacement is constant with respect to the SRS designs and independent of sample size. Relative cost for the SRS/ratio estimation design is always 1 because selection is by SRS. In contrast, the expected total size of n selected primary units will not be strictly proportional to sample size when units are selected by PPS without replacement. Because all π_i are bounded by 1, relative cost for the PPS without replacement design is a decreasing function of sample size.

Changes in Elemental Composition of Hatchery-Reared Rainbow Trout, *Salmo gairdneri*, Associated with Growth and Reproduction

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By examining a group of rainbow trout (*Salmo gairdneri*) over their life cycle (ova to 1500 g), I found that their elemental composition was determined by fish size, stage of life cycle (prefeeding, juvenile, post-juvenile), and reproductive state. Fish were fed practical diets and were reared under hatchery conditions. Whole body elemental concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr, and Zn were size dependent prior to sexual maturity. Rates of elemental accumulation in relation to weight gain were higher in juveniles than in adult fish. Reduced somatic concentrations of Mn, Fe, and Zn were observed during gonad maturation in female but not in male trout. Tissue concentrations of some elements remained constant over the duration of the study, while others increased or decreased linearly with increasing fish size. Tables and equations I present will enable the normal tissue and whole body elemental composition of rainbow trout at any size to be determined. My results indicate that body burden or wet weight concentration are better indicators of elemental status than dry weight concentration and that comparison of elemental levels between treatment groups in dietary experiments should be made on the basis of a standard-sized fish or by comparing the rates of elemental deposition with growth.

En examinant un groupe de truites arc-en-ciel (*Salmo gairdneri*) au cours de leur cycle vital (de l'œuf à 1500 g), j'ai constaté que leur composition élémentaire était fonction de la taille, de la période du cycle évolutif (stades pré-alimentaire, juvénile et post-juvénile) et de la situation reproductive. Les poissons étaient nourris de la façon habituelle et élevés dans les conditions ordinaires des piscicultures. Les concentrations en éléments de l'organisme entier étaient fonction de la taille avant la maturité sexuelle pour les éléments Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr et Zn. Les taux d'accumulation des éléments par rapport au gain de masse étaient plus élevés chez les juvéniles que chez les adultes. On a observé des concentrations somatiques réduites de Mn, Fe et Zn lors de la maturation des gonades chez la femelle, mais pas chez le mâle. Les concentrations dans les tissus pour certains éléments sont restées constantes pendant toute la durée de l'étude, alors que d'autres ont augmenté ou diminué de façon linéaire avec l'augmentation de la taille. Les tableaux et les équations que je présente permettront de déterminer la composition élémentaire normale des tissus et du corps entier de truites arc-en-ciel de n'importe quelle taille. Les résultats montrent que la charge corporelle ou la concentration par rapport à la masse humide sont de meilleures indicateurs de la composition élémentaire que la concentration par rapport à la masse sèche et que la comparaison des concentrations d'éléments entre des groupes soumis à des expériences alimentaires doit être effectuée pour des poissons de taille standard ou en comparant les taux de dépôt d'éléments en fonction de la croissance.

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Recent studies examining the dietary elemental requirements of fish have shown that depressed whole body or tissue levels of essential elements may result from insufficient dietary intake (Lovell 1978; Ogino and Yang 1978, 1980; Wilson et al. 1982; Gatlin et al. 1982). Deviations from normal whole body or tissue levels may be detectable before physiological dysfunction is observed (Underwood 1977). At present, insufficient data exist on normal levels. If measurements were available, comparing elemental levels in fish being raised under production conditions to established normal levels may provide a useful means of detecting dietary elemental inadequacies before fish health is impaired. Mertz (1970) suggested that whole body and tissue levels of some elements change during an animal's life cycle. My study was designed to determine normal whole body and tissue levels of several elements in hatchery-reared rainbow trout (*Salmo gairdneri*) and to determine if these levels changed with growth, stage of life cycle, or reproductive state.

Materials and Methods

Fish, Feeding, and Sampling

Rainbow trout were obtained from a research stock maintained by the University of Washington School of Fisheries. Two consecutive year-classes from the same parent stock were examined simultaneously. Examination of the 0+ age group began when unfertilized eggs were collected in January 1981 and continued for 14 mo; the average fish then weighed 114 g. These fish were reared in circular fiberglass tanks (76-cm diameter × 46-cm deep) supplied with lake water (Table 1).

The 1+ age fish were first sampled in April of 1981. The initial sample of fish was taken from a 12-m diameter × 70-cm

TABLE 1. Elemental concentration of rearing water and protein (%), fat (%), and elemental (μg/g) concentrations of practical diets fed to rainbow trout (ND = no data; NQ = not quantifiable).

Component[a]	Hatchery water	Diet (sampling point)[b]								
		D[c]	E	G	H	I	K	M	O	Q
Protein		43.0	43.0	48.0	48.0	38.0	38.0	38.0	38.0	40.0
Fat		12.0	12.0	9.5	9.5	9.5	9.5	9.5	9.5	10.0
Al	0.048	ND	ND	99	96	88	83	95	70	79
Ca	9.91	25 000	14 000	41 600	36 000	31 500	36 000	35 000	34 400	37 000
Co	NQ	0.67	0.43	1.26	1.18	0.79	NQ	0.61	0.50	1.30
Cr	NQ	NQ	NQ	1.35	2.25	NQ	NQ	1.49	NQ	NQ
Cu	NQ	3.68	5.83	21.0	35.5	22.7	19.0	12.6	29.8	12.3
Fe	0.086	346	277	428	357	420	373	355	351	456
K	0.484	10 050	5 080	9 900	10 450	10 460	10 670	9 780	9 640	11 680
Mg	4.06	1 490	1 880	3 030	3 960	3 200	3 400	3 060	3 580	3 280
Mn	NQ	14.2	18.8	62.3	101	67.4	121	63.5	88.5	71.0
Na	5.63	6 860	9 000	6 740	5 050	5 230	4 710	5 370	5 360	5 330
P	0.579	20 200	11 700	25 600	23 600	20 900	21 800	22 200	21 800	23 200
Sr	0.049	76.0	113	94.6	68.0	69.0	65.3	66.6	61.8	74.6
Zn	0.016	158	198	119	168	119	168	105	150	115

[a]Proximate composition based on manufacturer's analysis.
[b]See Fig. 1 for time of sample collection.
[c]University of Washington starter diet.

deep concrete circular pond containing approximately 1000 fish. From this group, 60 fish were subsequently transferred to a separate pond from which samples were drawn. Progeny from the 1+ age fish were reared for approximately 140 d under conditions similar to that of the 0+ age group.

At first feeding, fry were fed the University of Washington hatchery diet four times daily to satiation (Table 1). After the fish reached a mean weight of 0.70 g, they were fed commercial closed formula diets (Table 1). The proximate composition of these diets varied in accordance with normal dietary recommendations (NRC 1981). During their first year, the 1+ fish had been fed diets having the same nutrient composition as those fed to the 0+ fish. Levels of essential elements, enhanced with a mineral premix, exceeded established dietary requirements for rainbow trout (Ogino and Yang 1978, 1980; Ogino et al. 1978; Ogino and Takeda 1978; Knox et al. 1981).

Fish were sampled approximately each time they increased in weight by 100% or bimonthly, so that a large number of samplings occurred when the fish were small. Fish were not fed for 2 d prior to sampling. With the exception of the spawners (collected March 1982) and the first sampling of the 1+ fish, the first 10 fish captured by dip net were selected for sampling. Initial samples of the 1+ fish were provided by hatchery personnel, and only fully mature fish were selected for the final sample. Fish were killed by a blow to the head and were weighed to the nearest 0.01 g; the caudal peduncle was severed to obtain blood samples. The fish were stored in plastic bags at −30°C until thawed and dissected for individual tissue analysis. All samples were processed within 2 wk of being collected. Individual tissue samples were taken from all fish larger than 10 g. The following tissues were analyzed: eye, a portion of the dorsal lobe of the caudal fin, scales, skin, and muscle from just below the dorsal fin, whole blood, liver, gonad, and pooled samples of eggs or fry. Fish <10 g were processed in their entirety; for larger fish, a subsample of whole fish minus gonads was analyzed.

Sample Preparation and Analysis

Organ and tissue samples were placed into acid-washed glass scintillation vials. The remainder of the fish was minced in a grinder with stainless steel blade and plastic hopper, and the whole fish, or in larger fish a sample of approximately 100 g, was dried in an aluminum tray at 105°C. A maximum 1-g sample of dried whole fish was used for elemental analysis. After dry weight was determined, the samples were charred and then incinerated at 550°C for 16 h. The ash was dissolved in a mixture of equal parts of concentrated HCl and HNO_3 and appropriately diluted with deionized water so that elemental concentrations remained within the analytical capabilities of the measuring instrument, an inductively coupled argon plasma emission spectrophotometer (Jarrell-Ash AtomComp, Fisher Scientific, Waltham, MA).

Elements I selected for analysis included two nonessential elements, Al and Sr, five major elements, Ca, K, Mg, Na, and P, and six trace elements, Co, Cr, Cu, Fe, Mn, and Zn. All concentrations are reported as micrograms per gram wet weight and total body amounts as micrograms unless otherwise stated.

Proximate analyses to determine protein, ash, and moisture levels were conducted using conventional procedures (AOAC 1975).

Relationships between fish weight and the body burden (total amount in the body) of each element were derived using log transformed data, following the method of Parker and Vanstone (1966) for comparing fish growth and changes in proximate composition.

I used Minitab® (Ryan et al. 1981) and the methods of Zar (1974) to test for significant differences between regression parameters. Unless otherwise stated, significance level is 5%.

Because I found no differences in proximate composition or elemental level attributed to sex before the later stages of maturation (fish age 689 d), I have pooled data from both sexes prior to this point.

Results

Growth and Proximate Composition

Examination of growth using a plot of log fish weight versus time indicated the presence of three distinct growth phases (Fig. 1; Table 2). Each growth phase, which corresponds to the growth "stanza" delineated by Brett (1979), consists of the period over which the rate of increase in log weight with time was constant. The existence of three stanzas (prefeeding, points A–C; juvenile, points C–J; post-juvenile, points J–Q) was further substantiated by examining data collected on proximate composition.

Percent dry matter content decreased in stanza 1, increased during stanza 2, and remained relatively constant during stanza 3 (Fig. 2). Measurements taken on other proximate components (data not presented) also suggest compositional differences between juveniles and post-juveniles.

Whole Body and Tissue Elemental Composition

Whole body Co concentrations were not quantifiable. Concentrations of Al and Cr showed large random variations between samplings. Concentrations of other elements changed considerably as the fish increased in weight. The degree and direction of these changes for some elements depended on whether the concentrations were expressed on a wet or dry basis (Fig. 3). The rate of uptake of each element in relation to the rate of body mass increase (log weight versus log body burden element) remained constant over a distinct size range (Fig. 4). These size ranges corresponded to the growth stanzas, with changes in the uptake rate occurring between points C and D and between points J and K. Regression analysis performed on data falling between inflection points (Table 3) indicates that all elements were taken up at a rate faster than the rate at which the fish added body mass ($b > 1$), or that the concentrations increased during the period represented by stanza 2, points C–J. In the following period (stanza 3), J to Q, concentrations of the elements either decreased or remained constant ($b \leq 1$).

Concentrations of the elements in the tissues I examined either remained constant over the study period or increased or decreased linearly as the fish increased in weight (Tables 4 and 5).

Sexual Maturation

Mean elemental body burdens of fish sampled at points R and S were not included in the regressions of log body burden versus log fish weight, since it was expected that maturation might affect these relationships. Observed body burdens of each element at points R and S were compared with burdens for fish of this size predicted by the stanza 3 regression equations of log body burden versus log fish weight (Table 3). Observed burdens of all elements were below predicted levels at point R. At point S, all burdens were below predicted levels and burdens of Fe, Mn, Sr, and Zn were significantly below predicted levels.

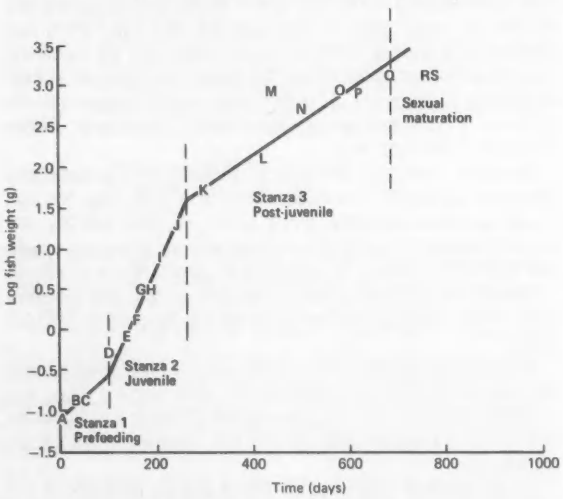

FIG. 1. Relationship between log fish weight and time for rainbow trout. The increase in log weight with time is linear over each growth stanza (regression parameters given in Table 2). Each point represents the mean of 10 fish. Point A = unfertilized egg; B = newly hatched fry; D = fish after first feeding; N = second sampling of 1+ fish; M = first sampling of 1+ fish.

TABLE 2. Growth of hatchery-reared rainbow trout from unfertilized egg to 1508 g. Log $\bar{X}$ fish weight (g) = $a + b$ (time in days). Each point represents the mean of 10 fish.

Stanza[a]	a	b	SD (b)	r^2	n	Time (d)
1	−1.09	0.0043	0.0015	0.886	3	1–82
2	−1.52	0.0115	0.0008	0.969	8	82–234
3	0.399	0.0042	0.0003	0.971	7	234–689

[a]Each stanza is the period during which the rate of increase in log weight with time is constant.

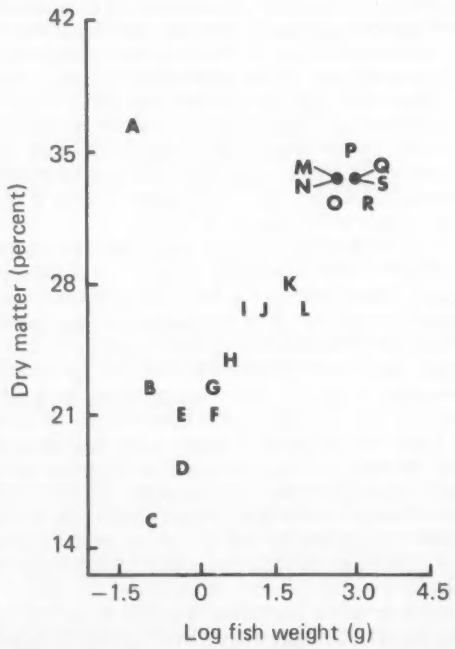

FIG. 2. Mean percent dry matter content (10 fish per sample) of rainbow trout from unfertilized egg to mature adult. Letters refer to age of fish (see Fig. 1).

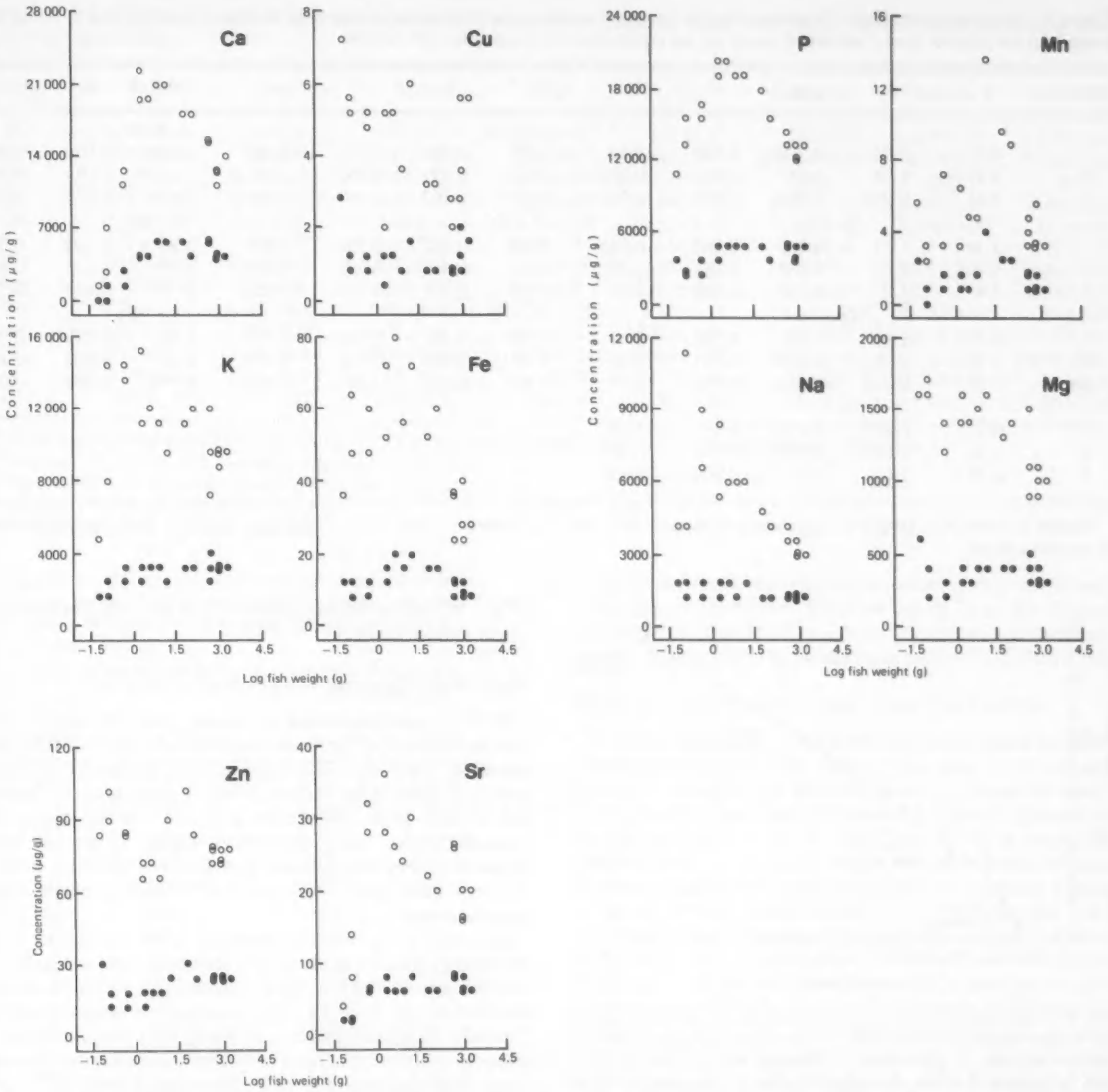

FIG. 3. Mean concentration of elements at selected points in the life cycle of hatchery-reared rainbow trout. ○, µg/g dry weight; ●, µg/g wet weight. Each point represents the mean of 10 fish.

The gonosomatic index (GSI) and the percentage dry weight of the ovary began to increase between points O and P and increased rapidly after point Q (Fig. 5). Concentrations of Ca, Mg, P, and Sr in the ovary increased throughout the maturation period, whereas concentrations of the other elements examined increased during early ovarian development and then declined as maturation approached (Fig. 5).

Maturation of male trout was not synchronous with that of females, as mature males were found during all samplings of fish >1 yr. Changes in the elemental composition of testes with maturation were, therefore, not examined. The whole body burdens of two mature male trout were examined at point S, and no element in either male differed significantly from predicted body burdens for fish of their size.

Discussion

Changes observed in the elemental composition of growing trout appear to be associated with specific life cycle stages as well as increasing body size. I identified four distinct stages: incubating eggs and prefeeding fry, juvenile, post-juvenile, and sexually maturing adult. Although changes in whole body and tissue elemental levels occurred throughout the fish's life cycle, they were most easily examined in relation to a specific life stage.

Elemental Composition of Eggs and Fry

Regression analysis of log egg and fry weight versus log elemental burden (Table 3) indicates that Ca was incorporated

TABLE 3. Regression parameters for determining the burden of an element in rainbow trout. Log body burden element (g) = $a + b$ (log fish weight) (g) for rainbow trout. Underlined values are not significantly different from 1 ($P < 0.05$).

Stanza[a]	a	b	SD	r^2	a	b	SD	r^2	a	b	SD	r^2
		Ca				*Cu*				*Fe*		
1	4.12	2.34	0.799	0.895	−2.16	−1.35	0.593	0.838	0.345	0.273	0.019	0.995
2	3.43	1.36	0.057	0.990	−0.052	1.12	0.091	0.962	1.08	1.17	0.044	0.992
3	3.81	0.950	0.024	0.997	0.055	0.990	0.102	0.949	1.46	0.856	0.038	0.988
		K				*Mg*				*Mn*		
1	3.59	1.31	0.113	0.993	0.846	−0.778	0.147	0.966	−3.84	−2.82	0.192	0.995
2	3.42	1.05	0.032	0.994	2.46	1.13	0.018	0.999	−0.086	1.39	0.106	0.967
3	3.43	1.03	0.042	0.990	2.66	0.968	0.038	0.991	0.853	0.760	0.043	0.981
		Na				*P*				*Sr*		
1	0.331	1.16	1.10	0.526	2.47	−0.037	0.091	0.140	0.751	1.48	0.079	0.997
2	3.19	0.986	0.028	0.995	3.55	1.18	0.020	0.998	0.702	1.20	0.065	0.983
3	3.19	0.965	0.034	0.994	3.75	0.965	0.022	0.997	0.838	0.999	0.057	0.984
		Zn										
1	−0.122	−0.482	0.135	0.927	−2.42							
2	1.20	1.08	0.033	0.994	−0.702							
3	1.39	1.01	0.016	0.998	−0.673							

[a]Stanza 1, points A–C (egg to 0.14 g); stanza 2, points C–J (0.14–17 g); stanza 3, points J–Q (17–1382 g). See Fig. 1 for further explanation of sampling points.

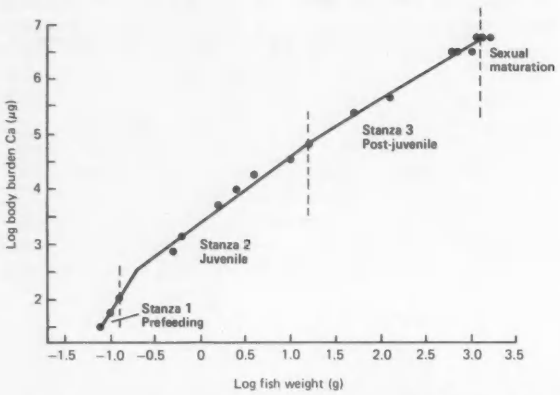

FIG. 4. Relationship between log fish weight and log body burden Ca. Linear regressions (see Table 3 for regression parameters) are shown for stanza 1, prefeeding; 2, juvenile; 3, post-juvenile. Each point is the mean of 10 fish. The largest coefficient of variation is <2%.

into the egg during incubation ($b > 1$). Regression analysis of data presented by McCoy et al. (1936) indicates an almost identical rate of calcium uptake in the eggs and fry of brook trout (*Salvelinus fontinalis*) despite differences in water quality. My data also indicate that Fe, K, Na, and Sr were assimilated during incubation ($b > 1$), while levels of Cu, Mg, Mn, P, and Zn declined ($b < 1$) (Table 3).

The elemental levels of the eggs and fry from the 1+ age group were not significantly different from levels observed in eggs or fry examined at the beginning of the study. Since the parents of both sets of eggs were fed identical diets, possibly the diet of the adults or the particular conditions under which these eggs and fry were reared led to identical compositions. A more likely alternative, in light of the similarity of elemental composition of the eggs and fry from this study to others (McCoy et al. 1936; Ogino and Yasuda 1962; Zeitoun et al. 1976), is that biological processes control egg composition within narrow limits.

Whole Body Elemental Composition in Juveniles

With the commencement of feeding (point D), whole body concentrations of all elements, with the exception of Na and K, increased (Table 3). This increase may be due to the rapid growth of some body organs during juvenile growth (Denton and Yousef 1976; Weatherly and Gill 1983a). Since the concentration of many elements is higher in specific body organs or tissues than in whole fish, positive allometric growth of these body parts will produce increasing whole body concentrations.

Increases in Ca levels in juveniles were identical to those reported by McCoy et al. (1936) for brook trout fed a diet of liver that was low in Ca. They indicated that sufficient Ca was obtained directly from the water to maintain normal whole body Ca levels. Relationships between whole body concentrations of other elements and size have been reported for various juvenile fishes (Bohn and McElroy 1976; Giesy and Wiener 1977). Dry weight concentrations were correlated with wet body weight or fish length, and observed declines, although significant, were small. Possibly, as with most juvenile fishes, the percent body fat of the fish examined in these studies increased as the fish grew, and a dilution effect occurred. The relative contributions of fat and defatted dry whole fish to the total body burden of some elements indicate that body fat tends to dilute elemental concentrations of some elements when they are reported on a dry weight basis (Table 6). Reporting results on a wet concentration or body burden versus fish weight basis may have produced different relationships.

Pierson (1981) reported large declines in Zn concentrations associated with growth in juvenile guppies (*Poecilia reticulata*), but initial Zn concentrations (up to 11 500 µg/g dry weight) appear high and declines in concentration appear to be greater than can be attributed to growth dilution.

Whole body concentrations of various elements in juvenile trout of a specific size have been reported (Ogino and Kamizono

TABLE 4. Grand mean and standard deviation of means of elemental concentrations (μg/g wet weight) found in tissues of rainbow trout[a] (NQ = not quantifiable).

Element	Blood	Liver	Scale	Skin	Eye	Caudal fin	Muscle	Whole fish
Al	0.58±0.26	22±17	17±24	8.6±9.4	3.8±7.5	16±16	2.7±1.8	2.3±1.1
Ca	109±43[d]	82±30[d]	31510±4931	736±507[d]	852±197	20990±9345[e]	116±35.7[c]	5164±1160
Co	NQ	0.29±0.24	0.59±1.07	0.62±0.85	0.12±0.15	NQ	NQ	NQ
Cr	0.96±1.93[e]	0.22±0.38[e]	1.8±1.7	1.6±1.9[e]	0.49±0.86	0.78±1.3[e]	0.23±0.14[e]	0.20±0.20
Cu	0.94±0.45[e]	101±41	3.0±2.4	1.3±0.84[e]	0.57±0.28	1.1±0.42	0.59±0.36[e]	1.2±0.46
Fe	302±41	89±55[e]	19±7.8[e]	13±11[e]	15±8.1	9.8±3.4[e]	3.7±1.0	12±3.8[e]
K	812±463[d]	3248±540	1632±690	914±840[c]	610±129	1605±537	3885±788[c]	3154±469
Mg	72±10.0	209±28	883±168	134±64[c]	91±26[c]	541±223[e]	322±27	333±163
Mn	0.22±0.12[e]	1.47±0.31	7.60±2.92[b,c]	0.41±0.20	0.30±0.10	5.0±1.8[c,e]	0.12±0.02	1.81±0.88
Na	2941±364[c]	1439±320[c]	2308±424	1951±304	2692±304	2301±593	661±129	1326±300
P	1315±83	3332±354	18054±3395	1457±606[c,d]	1094±117[c]	12401±3312[e]	2472±231	4804±963
Sr	0.17±0.09[e]	0.14±0.12[e]	55±15	1.2±0.90	1.3±0.41	33±13[e]	0.10±0.05[e]	7.4±2.60
Zn	13±3.0[e]	24±4.0[d]	47±11[e]	23±9.4[c]	53±7.5[c]	33±7.6[e]	4.4±1.0	25±1.60

[a]Mean and standard deviation of 95 fish (10–1822 g): males and females pooled up to 689 d; females only thereafter.
[b]Increase ($P < 0.05$) with increasing fish size.
[c]Decrease ($P < 0.05$) with increasing fish size.
[d]Increase ($P < 0.05$) during maturation.
[e]Decrease ($P < 0.05$) during maturation.

TABLE 5. Tissues of rainbow trout in which elemental concentrations (μg/g wet weight) change with fish growth. Relationships are linear and significant at the 5% level, $n = 75$.

		Fish weight (g)	
Element	Tissue	16.98	1382
Cu	Whole blood	2.0	0.51
	Skin	3.1	0.50
K	Whole blood	77	1269
	Skin	2291	219
	Muscle	4900	3084
Mg	Skin	275	94
	Eye	153	65
Mn	Scales	4.1	11.4
	Caudal fin	3.2	7.4
Na	Liver	1850	1093
	Whole blood	3705	2381
P	Skin	2826	1213
	Eye	1337	998
Zn	Eye	58	48
	Skin	38	15

1975; Ogino and Yang 1978) and concentrations are similar to those I found for similar sized fish despite large differences in dietary elemental concentrations and rearing conditions. This similarity in body concentrations suggests that the incorporation into the body of essential elements from dietary and waterborne sources is closely regulated.

Cutshall et al. (1977) examined the relationship between size and wet weight Zn concentration in Pacific hake (*Merluccius productus*) as they made the transition from juvenile to adult. They reported that Zn concentrations increased with increasing size in juveniles and remained relatively constant in adults. They attributed this decline in the rate of Zn uptake to lower levels of Zn in the diet of adult fish. In my study, the dietary Zn concentration remained relatively constant during the juvenile to post-juvenile transition, while the rate of Zn uptake declined significantly (Table 3). Whole body concentrations, therefore, appear to be controlled by homeostasis rather than dietary input.

Whole Body Elemental Composition in Post-Juveniles

A change in the rate of elemental uptake in relation to growth occurred at approximately point J (fish age 234 d) for each element I examined with the exception of K, where the rate of uptake remained unchanged. Values of *b* (Table 3) indicate that the concentrations of Cu, K, Mg, Sr, and Zn in whole fish remain constant in stanza 3, that is, the body burden of these elements increased at a rate equivalent to the increase in body weight of the fish. Concentrations of Ca, Fe, Mn, Na, and P decreased. None of the elements examined increased in concentration during post-juvenile growth. Weatherly and Gill (1983a) indicated that during post-juvenile growth, organs tend to grow at a rate slower than the carcass. The outcome of this negative allometry is declining whole body concentrations of some elements. Isometric growth would lead to an unchanging concentration as the fish increased in size. The relationships between fish size and elemental composition in post-juvenile or adult fishes have been examined previously (Goodyear and Boyd 1972; Tong et al. 1974; Wiener and Giesy 1979). These studies examined the elemental concentrations of wild fish. A study by Wood et al. (1957) indicated that, at least for salmonids, protein and fat levels can differ considerably between wild fish and fish raised under hatchery conditions. This difference in body composition influences the apparent elemental concentrations of the fish if they are reported on a dry weight basis, and may have contributed to differences between these and my study.

Tissue Elemental Concentrations

Declines in muscle concentrations of Fe in bluefish (*Potamus saltatrix*) and Cu and Zn in morids (*Antimora rostrata*) attributed to dilution by growth, changes in proximate composition of muscle, or decreased dietary intake were reported by Cross et al. (1973). In my study, concentrations of all elements

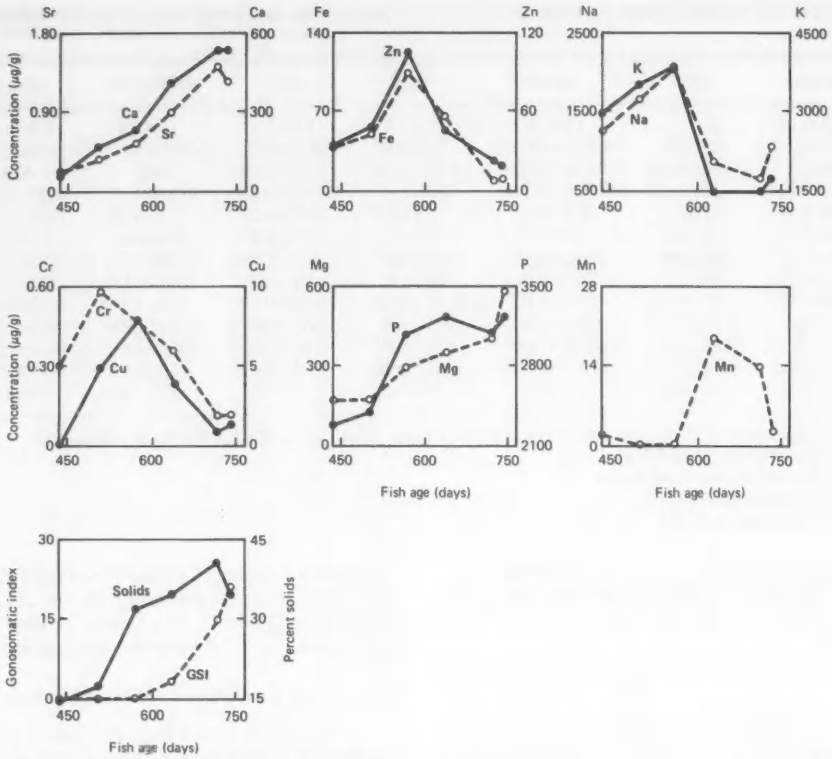

FIG. 5. Concentrations (µg/g wet weight) of elements in ovary of rainbow trout, gonosomatic index (GSI), and percent dry matter of ovary. Each point represents the mean of 10 fish. Standard errors fall within the area of the circles.

TABLE 6. Contribution of body fat to the total elemental burden (µg) of a rainbow trout[a] (ND = not detectable).

Element	Fraction[b] Fat (1)	Defatted whole fish (2)	Whole fish (1 + 2)	% of element in fat (1)/(1 + 2)
Al	0.60±0.78	3.00±2.88	3.60±3.66	16.6
Ca	8.23±6.94	15 013±1818	15 221±1825	0.05
Co	ND	0.016±0.028	0.016±0.028	ND
Cr	ND	0.75±0.126	0.75±0.126	ND
Cu	0.102±0.077	3.68±1.43	3.78±1.51	2.70
Fe	0.09±0.04	19.26±2.87	19.35±2.91	0.46
K	9.58±5.65	8128±570	8138±576	0.12
Mg	0.89±0.31	998±32	999±32	0.09
Mn	ND	3.08±0.96	3.08±0.96	ND
Na	39±26	2751±133	2790±159	1.40
P	134±20	12 406±719	12 540±739	1.07
Sr	0.014±0.013	21.44±2.21	21.45±2.22	0.07
Zn	1.76±2.71	58.98±16.40	60.74±19.11	2.89
Weight (g)	0.145	0.855	1.00	

[a]Fat extracted in a Goldfisch fat extractor using dichloromethane.

[b]Mean proximate composition of three fish (dry weight basis); protein 71.3%, fat 14.5%, ash 14.0%. Fish sampled at point R; weights 1822, 1367, and 1608 g.

except K, in the muscle of fish >10 g, remained constant despite increasing fish weight and change in proximate composition. Wiener and Giesy (1979) reported similar findings in wild bluegill (*Lepomis macrochirus*), as did Knox et al. (1981) for cultured trout.

Liver elemental concentrations agree closely with those reported by Knox et al. (1982) and Wiener and Giesy (1979), but differ considerably from those reported by Tacon (1978) who fed trout diets containing activated sewage sludge that had levels of several elements that may have been high enough to overcome homeostasis. Ogino and Yang (1978) reported lower levels of Cu in the livers of small trout, but the diets they fed were lower in Cu (0.7 and 3.0 μg/g) than in my study (3.7 to 35.5 μg/g).

During sexual maturation, liver levels of Ca and Zn increased, and levels of Cr, Fe, and Sr decreased significantly. These changes were not observed by Fletcher and King (1978) who examined the composition of sockeye salmon (*Oncorhynchus nerka*) livers during maturation. Shackley et al. (1981) reported that liver Cu was transported to the ovary of the blenny (*Blennis pholis*) during maturation, but this process was not evident in my study.

Eye Fe and Cu concentrations I observed agree closely with those reported by Ogino and Yang (1978) for similar sized fish. Significant declines in eye P, Mg, and Zn concentrations as the fish grew were possibly due to dilution by material containing low levels of these elements, as the eye changed considerably in percent dry matter as the fish grew (16.6–32.2%, points J–S).

Declines in scale Fe, Mn, and Zn during maturation have previously been observed in sea trout (*Salmo trutta*) (O'Grady 1981). Muguja and Watabe (1977) reported mobilization of scale Ca in carp (*Carassius auratus*) during maturation. In my study, Ca was apparently obtained from areas other than the scales (Table 4).

Sexual Maturation

Calcium, Mg, P, and Sr increased in concentration in the ovary throughout maturation (Fig. 5). The other elements, Cr, Cu, Fe, K, Mn, Na, and Zn, increased in concentration during early maturation (up to point O, day 570) and then declined rapidly as the GSI increased. These changes have been reported to occur in the blenny and sockeye salmon (Fletcher and King 1978; Shackley et al. 1981) and indicate a need for specific elements at a particular stage of ovarian development.

During maturation the elements showing the greatest change in concentration in the tissues examined (Fe, Mn, Sr, and Zn) were present in the whole body at less than levels predicted by the stanza 3 weight versus body burden relationship. This decline may be due to a normal transfer of elemental stores to the ovary. Alternatively, dietary concentrations of Fe, Mn, and Zn may have been lower than that required by maturing female trout. Takeuchi et al. (1981) demonstrated that when maturing trout were fed diets deficient in Mn, Zn, Cu, and Fe, the levels of Zn, Mn, and Fe were lower in the bones of the females at maturation than in the controls, while only Mn was lower in the eggs of the controls. When an elemental deficiency occurs, normal egg concentrations may be maintained at the expense of the somatic tissues. Low body burdens of Sr, an element regarded as nonessential (Underwood 1977), may have been associated with the transfer of somatic Ca to the ovary.

The elemental composition of a fish can be modified by feeding an unbalanced diet (Ogino and Takeda 1978; Ogino et al. 1978; Ogino and Yang 1980; Knox et al. 1981; Wilson et al. 1982; Gatlin et al. 1982) but comparison of elemental data collected in the current study with the published values referred to throughout this report indicates that homeostasis maintains body and tissue levels of the elements examined, within specific limits. This appears to be achieved by variable retention of dietary elemental input and assimilation of some elements directly from the water (NRC 1981). Weatherly and Gill (1983a) showed this tendency toward conservation of composition by demonstrating that for a given sized fish, the relative weights of body parts tend to be constant, regardless of the fish's previous rate of growth. This was also demonstrated for proximate composition by Reinitz (1983) and Weatherly and Gill (1983b).

The fish I examined were drawn from a population exhibiting apparently normal growth and reproduction. I assumed, first, that the diets fed were nutritionally adequate and, second, that the elemental concentrations (Table 4) and accumulation rates (Table 3) represented those of normal fish. Given these assumptions, these values can be used to help determine if dietary elemental levels are adequate.

In summary, this study has shown that trout size, life cycle stage, and, in female trout, state of sexual maturation must be considered before an abnormal elemental composition is confirmed. Whole body wet weight concentration and body burden appear to be more useful measurements for comparison than dry weight concentration, because variation in the amount of fat present in fish will alter dry weight concentration of some elements. Individual tissue concentrations may be useful indicators of deficiency for some elements, but fish size and reproductive state also determine normal tissue level.

One consequence of size-dependent elemental composition is that direct comparison between treatment groups in dietary experiments may yield misleading results if the treatments produce differential growth. This can be overcome by comparing the rates of elemental deposition between treatment groups (b values) or by adjusting the elemental levels so they are reported on the basis of a standard-sized fish. These comparisons or adjustments are only valid, however, if the fish are within the same life cycle stage.

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Extraction of Algal ATP and Interpretation of Measurements

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Hendzel, L. L., and F. P. Healey. 1984. Extraction of algal ATP and interpretation of measurements. Can. J. Fish. Aquat. Sci. 41: 1601–1608.

We compared ATP samples collected by direct injection with those collected by filtration from exponential and P-limited algal cultures, and from summer populations of several lakes. The direct injection method yielded higher amounts of measurable ATP than did filtration on 2.5- and 4.5-cm diameter membrane filters. ATP lost to the filtrate was negligible. Variation in ATP content with increasing nutrient deficiency was measured on six cultures. ATP/C decreased with N deficiency and to a greater degree with P deficiency. Large changes of ATP/C usually occurred with little change in viability of the cells. Short-term (1 h) nutrient enrichments generally did not change the ATP/C ratio. Phosphorus deficiency tended to increase the chlorophyll/ATP ratio and N deficiency to decrease it. We found that the short- and long-term effects of alkaline phosphatase on ATP extracts from P-sufficient and P-deficient *Scenedesmus quadricauda* were negligible. There appeared to be little effect on frozen ATP samples stored up to 60 d. Any short-term effects were similarly negligible, since temperature and the addition of P did not affect the rate of ATP loss. The use of 0.33 mol $H_3PO_4 \cdot L^{-1}$ as an extractant proved to be completely unreliable.

Nous avons comparé des échantillons d'ATP obtenus par injection directe à ceux recueillis par filtration d'algues cultivées en présence d'un apport en P exponentiel et en milieu où l'apport en P était limité et de populations estivales d'algues de plusieurs lacs. La méthode d'injection directe a produit des quantités d'ATP mesurable supérieures à celles obtenues par filtration sur membranes filtrantes de 2,5 et 4,5 cm de diamètre. La quantité d'ATP perdue avec le filtrat était négligeable. Les variations de la teneur en ATP en fonction d'une carence nutritive croissante ont été mesurées avec six cultures. Le rapport ATP/C diminuait proportionnellement à la carence en N et la baisse était encore plus marquée dans le cas de la carence en P. Les grandes variations du rapport ATP/C survenaient habituellement sans qu'il y ait de grand changement dans la viabilité des cellules. En général, le fait d'enrichir à court terme (1 h) le milieu avec des éléments nutritifs ne modifiait pas le rapport ATP/C. La carence en phosphore tendait à augmenter le rapport chlorophylle/ATP, tandis que la carence en N tendait à le diminuer. Nous avons constaté que les effets à court et à long termes de la phosphatase alcaline sur les extraits d'ATP provenant de *Scenedesmus quadricauda* poussant en milieux suffisants en P et carencés en P, étaient négligeables. On a trouvé peu d'effet sur les échantillons d'ATP congelés et conservés pendant une période allant jusqu'à 60 jours. De la même façon, les effets à court terme étaient négligeables, car ni la température ni l'addition de phosphore n'influaient sur la vitesse d'élimination de l'ATP. L'utilisation de 0,33 mol $H_3PO_4 \cdot L^{-1}$ comme agent d'extraction n'était aucunement fiable.

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Initial interest in measuring the ATP content of plankton arose from its possible use as an indicator of living microbial biomass (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967; Holm-Hansen 1969, 1971; Holm-Hansen and Paerl 1972). ATP was believed to be associated only with living biomass because of its lability. However, this lability may also bring disadvantages. We investigated two of these potential problem areas: the loss of ATP during sampling and extraction, and the constancy of ATP/C ratios with changes in physiological state.

Planktonic ATP samples are usually collected by filtration, followed by extraction of the filters in a boiling aqueous Tris (tris(hydroxymethyl)aminomethane) solution (Holm-Hansen and Booth 1966; Holm-Hansen 1969; Rudd and Hamilton 1973; Cavari 1976; Jones and Simon 1977; Perry et al. 1979). Because of the possibility of cell breakage during filtration and of ATP degradation during extraction of the filter, filtration was compared with direct pipetting into boiling Tris buffer, using both algal cultures and summer lake plankton samples.

The use of ATP as a measure of living microbial biomass is dependent on a constant ATP/C ratio in living microbes. Early studies indicated this to be true for exponentially growing algae and bacteria, but also showed that during nutrient deficiency this ratio could be very depressed (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967). Since then, other studies have shown depressed ATP/C ratios with nutrient deficiency (Holm-Hansen 1970; Bottomley and Stewart 1976; Cavari 1976; Healey 1979), not a surprising result considering the metabolic lability of ATP and the great variations in microbial composition and metabolism that can be induced by changes in physiological state (Herbert 1961; Healey 1975). We have looked at variations in ATP/C during the growth of cultures of several algae into P and N deficiency. We have also attempted to see if there was any relationship between changes in the ATP/C

ratio and changes in viability during growth into P and N deficiency in order to determine if depressed ATP/C ratios in nutrient deficiency could be due to loss of viability.

Methods

Culturing

The algae used in this study were *Chlamydomonas reinhardi* Dangeard (3), *Scenedesmus quadricauda* (Turp.) de Breb. (11), *Ankistrodesmus braunii* (Naeg.) Brunnthaler (18), *Anabaena variabilis* Kuetz. (21), *Cryptomonas erosa* Ehrenberg (40), *Ochromonas vallesiaca* Chodat. (53), *Pseudoanabaena catenata* Lauterb. (57), *Chlamydomonas* spp. (81 and 94), *Navicula pelliculosa* (Breb.) Hilse. (85), *Melosira* sp. (95), *Fragilaria* sp., and *Synura sphagnicola* Korsch. The numbers in parentheses refer to the culture collection at the Freshwater Institute. Except for *O. vallesiaca*, the first 10 cultures were axenic. The cultures were grown at 20°C, either in large Pyrex test tubes of 1.0-L volume, bubbled with air, and lighted from one side by Vita-Lite fluorescent tubes providing an intensity of 50 μEinsteins$\cdot m^{-2}\cdot s^{-1}$ at the tube surface (Healey 1973) or in 500-mL Nephelo culture flasks (Bellco Glass Co.) containing 200 mL of medium on an orbital shaker lighted from above by Vita-Lite fluorescent tubes at an intensity of 50 μEinsteins$\cdot m^{-2}\cdot s^{-1}$ at the top of the shaker. *Synura sphagnicola* was grown at 10°C in 500 mL of medium in 2.8-L Fernbach flasks, lighted from below at an intensity of 20–25 μEinsteins$\cdot m^{-2}\cdot s^{-1}$ at the bottom of the flasks.

All species except *S. sphagnicola* and *Melosira* sp. were cultured in modifications of WC medium (Guillard and Lorenzen 1972). Complete WC medium was only slightly modified by replacing Tris buffer with Bicine (*N,N*-bis(2-hydroxyethyl)glycine) buffer. Phosphorus-limited WC medium had K_2HPO_4 replaced with equimolar KCl and KH_2PO_4 added to a final concentration of 1–10 μmol $P\cdot L^{-1}$. The N-limited WC medium had $NaNO_3$ replaced with equimolar NaCl and a final concentration of 25–100 μmol $NaNO_3\cdot L^{-1}$. *Fragilaria* sp. was grown in a further modification of WC medium, where $NaNO_3$ was decreased to 200 μmol $\cdot L^{-1}$, $Na_2SiO_3\cdot 9H_2O$ was increased to 200 μmol$\cdot L^{-1}$, trace element solution was reduced by one-half, and the buffer was 4.0 mmol Tris$\cdot L^{-1}$ (pH 7.5). The low-P medium had K_2HPO_4 replaced with equimolar KCl, and KH_2PO_4 was added to give 5 μmol $P\cdot L^{-1}$, whereas in the low-N medium, $NaNO_3$ was replaced with equimolar NaCl, and $NaNO_3$ was added to give 50 μmol $N\cdot L^{-1}$. *Melosira* sp. and *S. sphagnicola* were grown in filtered water from Lake 226 (Experimental Lakes Area, Schindler and Fee 1974), enriched with 200 μmol $Na_2SiO_3\cdot 9H_2O\cdot L^{-1}$ and trace elements and vitamins at 20% of the level in medium WC. The complete medium contained 200 μmol $NaNO_3\cdot L^{-1}$ and 10 μmol $KH_2PO_4\cdot L^{-1}$, the N-limited medium contained 10 μmol $NaNO_3\cdot L^{-1}$ and 10 μmol $KH_2PO_4\cdot L^{-1}$, and the P-limited medium contained 200 μmol $NaNO_3\cdot L^{-1}$ and 1.0 μmol $KH_2PO_4\cdot L^{-1}$.

Generally, growth was followed by measuring optical density at 750 nm on a Bauch & Lomb Spectronic 20 or 100. Growth of *Melosira* and *Synura* cultures was monitored by microscopic cell counts.

ATP Extractions

Using exponential and P-limited cultures of eight algae cultured in the Nephelo flasks, collection of ATP samples by direct pipetting was compared with that by filtration (Fig. 1). Subsamples of 1.0 mL were filtered onto 4.5- and 2.4-cm diameter, 0.45-μm pore size membrane filters (Sartorius) with mild vacuum (12 cm Hg). Upon completion of filtration, the filters were immediately placed in a beaker of the appropriate diameter (Pyrex 100 mL for the 4.5-cm filter and 20 mL for the 2.4-cm filters) at room temperature; approximately 5.0 mL of boiling Tris buffer (0.05 mol$\cdot L^{-1}$, pH 7.7) was injected onto the filter; then the beaker and filter were placed in a boiling water bath for 5 min. In addition, with four of the algae (*S. quadricauda*, *C. reinhardi*, *A. variabilis*, and *P. catenata* grown on complete and P-limited WC medium) a second set of samples was filtered onto 4.5-cm, 0.45-μm membrane filters. Upon completion of filtering, the filters were immediately placed into a 100-mL beaker containing 5.0 mL of boiling Tris buffer held in a boiling water bath. Also, subsamples of the same volume were directly pipetted into 4.0 mL of boiling Tris buffer and held in a boiling water bath for 5 min. Immediately following extraction, the volume of the extract was adjusted to 5.0 mL with Tris buffer (0.05 mol$\cdot L^{-1}$) and then the extract was rapidly frozen at −17°C. Filtrate from the samples filtered onto the 4.5- and 2.4-cm membrane filters was collected in test tubes containing approximately 4.0 mL of boiling Tris buffer held in a 500-mL vacuum flask on a hot plate with boiling water sufficient to keep the 5.0-mL extract boiling. After the filtration was completed and the filter extracted, the test tube containing the filtrate was transferred to a boiling water bath for 5 min, then made to 5.0 mL, and rapidly frozen in a manner similar to the filter extracts. All ATP samples were collected in triplicate and subsequent analysis followed the procedure of Rudd and Hamilton (1973). The mean standard deviation of triplicate samples was ±12% for both extraction methods.

Cultures of *S. quadricauda*, *A. braunii*, *Chlamydomonas* spp. (81 and 94), *Fragilaria* sp., and *S. sphagnicola* grown into P or N deficiency were used to measure the amount of variation in ATP content with increasing N or P deficiency. *Synura sphagnicola* was also grown with no nutrient deficiency. Concentration of suspensions by centrifugation was needed for several cultures only during logarithmic growth when OD_{750} was less than 0.1. Suspensions for ATP extraction were placed in the light for 30 min. The first set of samples was collected in triplicate by pipetting 1.0-mL aliquots into 4.0 mL of boiling Tris buffer (0.05 mol$\cdot L^{-1}$, pH 7.7), maintained in a boiling water bath for 5 min, and then made to 5.0 mL as above and rapidly frozen at −17°C. Additions of 5 μmol $P\cdot L^{-1}$ as KH_2PO_4 or 50 μmol $N\cdot L^{-1}$ as NH_4Cl were made to the suspensions; after 1 h in the light (Vita-Lite, 166 μEinsteins$\cdot m^{-2}\cdot s^{-1}$), a second set of samples was pipetted.

Effect of Alkaline Phosphatase on Potential Loss of ATP

To determine whether alkaline phosphatase could contribute to the lowered ATP/C ratios of samples from P-deficient cultures, we followed long- and short-term loss of ATP from extracts of P-sufficient and P-deficient cells.

Scenedesmus quadricauda was grown in complete and low-P (10 μmol $P\cdot L^{-1}$) WC medium in 2.8-L Fernbach flasks. Five triplicate sets of samples were extracted by pipetting 1.0-mL aliquots into approximately 4.0 mL of boiling Tris buffer. Following extraction these were adjusted to 5.0 mL with Tris buffer (0.05 mol$\cdot L^{-1}$) and frozen. This was done three times. In addition, a similar set of samples was extracted with 0.33 mol $H_3PO_4\cdot L^{-1}$ and 48 mmol EDTA$\cdot L^{-1}$, as suggested by Karl and

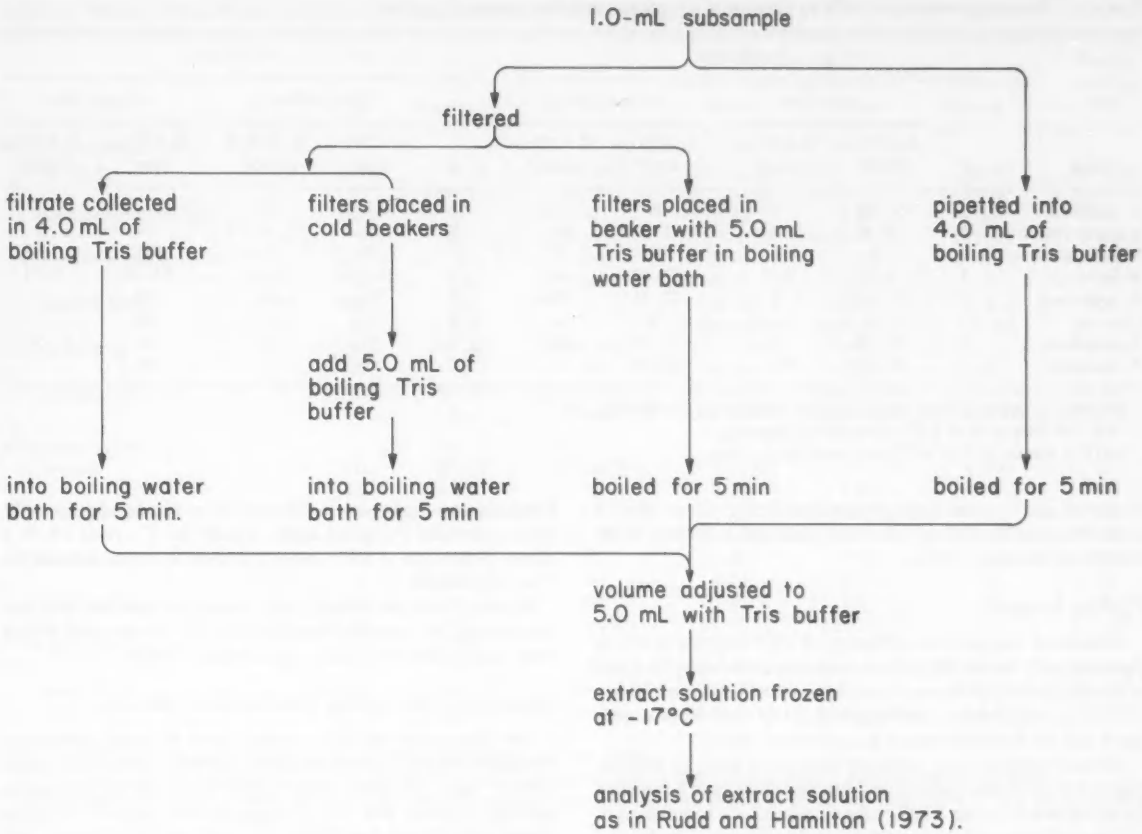
1.0-mL subsample
filtered
filtrate collected in 4.0 mL of boiling Tris buffer
filters placed in cold beakers
filters placed in beaker with 5.0 mL Tris buffer in boiling water bath
pipetted into 4.0 mL of boiling Tris buffer
add 5.0 mL of boiling Tris buffer
into boiling water bath for 5 min.
into boiling water bath for 5 min
boiled for 5 min
boiled for 5 min
volume adjusted to 5.0 mL with Tris buffer
extract solution frozen at -17°C
analysis of extract solution as in Rudd and Hamilton (1973).

FIG. 1. Summary of the ATP extraction procedures used.

Craven (1980) as a means of reducing possible hydrolysis of ATP by phosphatases. To remove a potential source of interference with luciferase, EDTA was omitted from one set of samples (Karl and LaRock 1975). One triplicate set of samples from the Tris extraction and the H_3PO_4 extraction was analyzed for ATP immediately and the remaining four sets of samples from each of the extraction methods were stored frozen at −17°C and analyzed over a period of 40–60 d.

Additional samples were extracted with Tris buffer and combined to yield 10-mL samples. One sample was kept at room temperature (21°C), while the second was held on ice (0°C). ATP was measured initially; this was followed by an addition of 10 μg ATP·L^{-1} to each sample. ATP was measured after the addition and then several more times for up to 2 h. To an extra sample extract from the P-deficient culture, we added 10 mmol P·L^{-1} along with the ATP addition and analyzed with time at room temperature. Finally, a control consisting of 10 mL of Tris buffer (0.05 mol·L^{-1}, pH 7.7) at room temperature received the same ATP addition and was analyzed along with the other samples. The P addition was increased to 100 mmol P·L^{-1} with the subsequent set of P-sufficient and P-deficient cultures.

Alkaline phosphatase, both soluble and cellular, was measured fluorometrically on the above cultures according to the method of Healey and Hendzel (1979).

Viability and Composition Measurements

During exponential and P- and N-limited growth of cultures of *S. quadricauda*, *A. braunii*, and *Chlamydomonas* spp. (81 and 94), samples were plated in triplicate on modified complete WC medium agar; double-strength medium and agar (1.4% Bacto-Difco agar) were autoclaved separately and combined prior to pouring the plates. Each plate received 50–100 algal units (i.e. single cells, paired cells, tetrads, etc.). Inoculated plates were then incubated at 20°C directly above Vita-Lite fluorescent tubes at an intensity of 30–50 μEinsteins·m^{-2}·s^{-1}. After 7–14 d, colonies were counted using a dissecting microscope at low power (15×). Plating efficiency (a minimum estimate of viability) was the number of colonies growing as a percentage of the units plated. We used only algae giving a high plating efficiency during exponential growth for this study. Viability of *S. sphagnicola*, from cultures of the same nutrient status as the previous cultures, was estimated as the percentage of colonies observed to be motile, whereas viability of *Fragilaria* sp. was estimated by counts of cells containing chloroplasts as determined by microscopic examination.

Particulate C, N, and P samples were collected on Whatman GF/C filters (2.4 cm) that had been preignited at 500°C for 12 h and analyzed as in Stainton et al. (1977). Chlorophyll samples were collected on GF/C filters (2.4 cm), extracted with

TABLE 1. Percentage recovery of ATP by filtration in comparison with that by direct pipetting.

	P-sufficient					P-deficient				
		2.5-cm filter		4.5-cm filter			2.5-cm filter		4.5-cm filter	
Alga	n[a]	% ATP on filter[b]	% ATP in filtrate[c]	% ATP on filter[b]	% ATP in filtrate[c]	n[a]	% ATP on filter[b]	% ATP in filtrate[c]	% ATP on filter[b]	% ATP in filtrate[c]
C. reinhardi	2	61–91	1	39	0.6	2	74–75	0	29–56	0
S. quadricauda	2	3–33	0	1–6	0	2	21	0	4	0
N. pelliculosa	1	71	2	98	2	1	79	3	108	0
Melosira sp.	3	71–78	0–2	67–97	0–2	3	28–76	0–8	55–80	0–11
O. vallesiaca	2	41–122	3	47–111	0.9	2	63	4	38	2
C. erosa	1	4	—	4	—	1	31	—	40	—
A. variabilis	2	55–98	0	30–71	0.3	2	51	0	32	0
P. catenata	2	75–128	0.5	65–94	2	2	80	0	50	0

[a]Number of cultures from which triplicate samples were collected.
[b]ATP on filter as % of ATP recovered by pipetting.
[c]ATP in filtrate as % of ATP recovered by pipetting.

methanol, and measured spectrophotometrically. Chlorophyll *a* in the presence of chlorophyll *b* was calculated according to the formula of Holden (1965).

Plankton Samples

In order to compare the collection of ATP samples by direct pipetting with that by filtration, we sampled 10 eutrophic lakes in the Erickson–Elphinstone area, Manitoba (Sunde and Barica 1975), one oligotrophic lake situated 25 km north of this area, and a bay on Southern Indian Lake (Hecky 1975).

Surface samples were collected from these lakes in 500-mL plastic bottles in the morning and returned to the field laboratory for extraction by noon. The Southern Indian Lake samples were refrigerated after collection and shipped to Winnipeg within 2 d for extraction.

Aliquots of 1.0 mL of unconcentrated lake water were filtered under mild vacuum (12 cm Hg) onto 2.4-cm, 0.45-μm membrane filters in triplicate, except for the oligotrophic lake sample where the volume filtered was increased to 50 mL. At the same time, 1.0-mL aliquots (including the oligotrophic lake) were pipetted directly into 4.0 mL of boiling Tris buffer held in a boiling water bath. The remainder of the extraction procedure followed that described for the laboratory cultures. Lake water from five of the eutrophic lakes was filtered (0.45-μm membrane filter) and approximately 25 mL of the filtrate was frozen in polyethylene vials for analysis of soluble ATP.

Composition samples consisting of particulate P and chlorophyll *a* were collected from each of these locations. Filtration and analysis were the same as described previously.

Results

ATP Extraction Methods

A comparison of ATP levels obtained by pipetting and by filtering onto 2.4- or 4.5-cm membrane filters was performed from one to three times on each culture shown in Table 1. In general, the amount of ATP recovered on the two sizes of membrane filters was only a fraction of that recovered by pipetting. The difference could not be found in the filtrate, where ATP was generally negligible, except for P-deficient *Melosira* sp. where up to 11% of the ATP was in the filtrate. Pipetting was superior to filtration to a similar degree with exponential and P-limited algae, except for *C. erosa* where a higher percentage of ATP was recoverable by filtration from the P-limited culture.

Heating both the beaker and extraction medium did not increase the percentage of recoverable ATP to any great extent over that obtained by using a cold beaker (Table 2).

Changes in ATP/C Ratios With Nutrient Deficiency

The algae listed in Table 3 were grown in media potentially limited by N or P to determine effects of these limitations on the ATP/C ratio. Measurements were made on exponentially growing cultures and were compared with those on cultures whose growth rate had fallen due to N or P deficiency. As supplementary indicators of N or P deficiency, the particulate N/C and P/C ratios were calculated for comparison with values associated with P and N deficiency in other algae (Healey 1975).

In general, exponential cultures and those whose growth rate was slowed by N deficiency had P/C ratios comparable with those of cells with no or only slight P deficiency. However, *S. quadricauda* had a surprisingly low P content when growing exponentially. A decrease in growth rate in low-P medium was accompanied by depressed P/C ratios, all to values indicative of severe P deficiency.

Exponentially growing cultures generally had N/C ratios that indicated no or only moderate N deficiency. However, low-P and low-N cultures generally had N/C ratios that were depressed. The one exception was *Chlamydomonas* sp. (94)

TABLE 2. Percentage recovery of ATP by filtration as affected by use of hot and cold beakers.[a]

	P-sufficient		P-deficient	
Alga	Cold beaker	Hot beaker	Cold beaker	Hot beaker
C. reinhardi	—	12	29	5
S. quadricauda	6	15	4	3
A. variabilis	71	13	32	17
P. catenata	65	90	50	69

[a]ATP on 4.5-cm filters as % of ATP recovered by pipetting.

TABLE 3. Effect of P and N deficiency on ATP content and plating viability of algae.

Alga	n[a]	μ (%)[b]	P/C (μg·mg^{-1})[c]	N/C (μg·mg^{-1})[c]	ATP/C (μg·mg^{-1})[c]		CHL/ATP (μg·μg^{-1})[c]	Plating viability (%)[d]
					Before	After		
P-limited medium								
A. braunii	3	100	4–22	101–168	0.35–1.29	0.61–1.71	30–85	96–138
	3	15	0.6–1	30–51	0.17–0.25	0.28–0.37	39–43	60–79
Chlamydomonas sp. (81)	1	100	21	167	0.90	0.80	35	98
	4	10	2–5	61–67	0.27–1.10	0.31–0.99	20–29	6–43
Chlamydomonas sp. (94)	1	100	19	143	2.30	2.30	10	21
	1	10	2	166	0.16	0.38	70	69
S. quadricauda	1	100	11	—	0.18	0.27	20	80
	3	10	1–2	—	0.006–0.026	0.08–0.13	8–82	78–105
Fragilaria sp.	2	100	17–20	114–141	1.82–1.91	1.76–1.80	11–15	99–100[e]
	1	10	5	64	0.13	0.12	45	95
S. sphagnicola	3	100	32–37	153–161	0.12–0.16	0.10–0.15	113–156	64–89[f]
	3	—	3–5	70–86	0.07–0.16	0.06–0.18	112–265	46–100
N-limited medium								
A. braunii	2	100	22–23	147–159	0.92–1.18	1.12–1.27	32–48	65
	1	10	22	73	1.24	1.13	10	2
Chlamydomonas sp. (81)	1	100	49	175	3.38	3.97	17	44
	2	10	49–62	69–76	0.87–1.63	1.18–1.59	8–16	38–63
Chlamydomonas sp. (94)	1	100	18	120	1.52	2.07	16	86
	1	10	18	52	0.61	0.78	8	11
S. quadricauda	1	100	—	190	1.07	1.15	24	86
	3	10	—	62–140	0.32–0.81	0.50–0.83	1–4	57–101
Fragilaria sp.	1	100	20	138	1.80	1.90	15	99[e]
	2	10	19–22	53	0.44–0.46	0.45–0.47	6–9	96–99
S. sphagnicola	3	100	32–37	153–161	0.12–0.16	0.10–0.15	113–156	64–85[f]
	3	17	38–41	27–56	0.05–0.10	0.05–0.09	94–307	57–95

[a] n = number of samples on which measurements of all parameters were made.
[b] μ = specific growth rates (based on cell numbers) as a % of maximum; 100% was growth rate before limiting nutrient depleted.
[c] Particulate ratios; ATP/C ratios are before and 1 h after addition of the limiting nutrient (5 μmol P·L^{-1} or 50 μmol N·L^{-1}); CHL = chlorophyll *a*.
[d] Number of colonies growing on agar plate as % of number of units (cells, colonies, or filaments) plated.
[e] Viability estimated as percentage of cells with chloroplasts.
[f] Viability estimated as percentage of colonies exhibiting motility.

growing on low-P medium, which showed a slight increase in N/C ratio with P deficiency.

Phosphorus and N deficiency had similar effects on ATP/C ratios. Phosphorus deficiency, in most cases, produced a marked depression of the ATP/C ratios except for the P-deficient culture of *Chlamydomonas* sp. (81), which showed little or no depression, and *S. sphagnicola*, which showed no significant change. Nitrogen deficiency generally depressed ATP/C ratios, but to a lesser extent than P deficiency. The exception in this case was *A. braunii* where there was a slight increase.

With several of the algae in Table 3, plating efficiency was used as a minimum estimate of viability to determine if the decline in ATP/C ratio with P or N deficiency could be attributed to loss of viability. There was no relationship between changes in ATP/C ratio and plating efficiency. Viability of N- and P-deficient *S. sphagnicola*, as determined by the presence of motility, remained higher than 50%, except for one experiment where it was about 50%. Motility of the nutrient-deficient cultures varied approximately as much as that in the exponentially growing, non-nutrient-deficient culture (Table 3). As with the other cultures, there was no relationship between the ATP/C ratio and motility. As for *Fragilaria* sp., where viability was estimated as a percentage of cells containing chloroplasts, there was little difference between cultures with or without P or N deficiencies; all cultures showed high viability (Table 3).

There are indications in the literature that the addition of P or N to P- or N-deficient algae may result in a rapid increase in ATP/C (Sakshaug and Holm-Hansen 1977; Healey 1979). With the exception of P-deficient *A. braunii* and *S. quadricauda*, this was not seen here.

Because P deficiency generally had a greater depressing effect on ATP/C ratios than did N deficiency and the reverse is often true for chlorophyll/C ratios, one might expect the ratio of chlorophyll/ATP to be higher in P deficiency than in N deficiency, as reported by Sakshaug and Holm-Hansen (1977) and Riemann and Wium-Andersen (1981). With the exception of some of the results with *S. sphagnicola*, this was generally seen here.

Effect of Alkaline Phosphatase on Potential Loss of ATP

Loss of ATP in unfrozen samples from *S. quadricauda* was not affected by P deficiency, by temperature, or by the addition of phosphate (Table 4). Phosphorus deficiency was shown to induce alkaline phosphatase activity in this alga (Healey and Hendzel 1975, 1979), and on the occasions when it was measured the expected alkaline phosphatase activity (soluble and cellular) was detected in the P-deficient cultures. Alkaline

TABLE 4. Short-term loss of ATP from samples of *S. quadricauda* following addition of 10 μg ATP·L^{-1}.

P status	Additions	Alkaline phosphatase activity (μmol OMF·L^{-1}·h^{-1})[a] Soluble	Cellular	Loss of ATP (μg ATP·L^{-1}·h^{-1}) 21°C	0°C
P-sufficient	None	0.035	0.18	5.0	4.9
P-deficient	None	1.94	4.35	4.6	4.3
	with 10 mmol P·L^{-1}	1.94	4.35	4.4	—
Blank (Tris buffer)	None	—	—	1.3	—

[a]OMF = *o*-methylfluorescein (Healey and Hendzel 1979).

TABLE 5. Measurement of ATP in natural seston samples.

Lake[a]	Chlorophyll (μg·L^{-1})	Particulate C (μg·L^{-1})	ATP (μg·L^{-1}) Pipetted	Filtered	Soluble[b]	% of pipetted ATP Filtered	Soluble
154	310	6900	14.62	7.09	0.15	49	1.0
302	12	4360	3.91	0.87	0.63	22	16
303	13	770	2.11	0.56	0.17	27	8.1
400	34	7260	7.24	1.93	0.38	27	5.2
885	157	3700	13.93	4.92	0.50	35	3.6

[a]Lakes in the Erickson–Elphinstone area (Sunde and Barica 1975).
[b]Soluble ATP is that passing through a 0.45-μm pore size membrane filter.

phosphatase activity was low in the P-sufficient culture. Insufficient phosphatase activity survives the boiling extraction procedure to affect ATP stability in the extracts. The addition of 100 mmol P·L^{-1} and 10 μg ATP·L^{-1} decreased measurable ATP by 20% relative to the addition of 10 mmol P·L^{-1} and 10 μg ATP·L^{-1}. This was followed by no change in the rate of loss of ATP with time, suggesting that some interference of the luciferase reaction occurred after the P addition.

During frozen storage at −17°C, loss of ATP over 60 d was small and similar whether the extracts were from a P-deficient or a P-sufficient culture.

The initial attempt to use 0.33 mol H_3PO_4·L^{-1} with 48 mmol EDTA·L^{-1} as the extracting medium resulted in light emissions from the first set of samples no higher than those for the blanks, whereas samples extracted with Tris buffer yielded 2.75 μg ATP·L^{-1}. For the remaining sets of samples the EDTA was deleted from the buffer, as its metal complexing qualities were not necessary with these laboratory samples. At 48 mmol·L^{-1}, EDTA was approaching the minimum concentation inhibitory to the luciferin–luciferase reaction (Karl and LaRock 1975). However, there was little improvement using H_3PO_4 without EDTA. All samples, with known amounts of ATP as confirmed by the Tris method, had light emissions no higher than the blanks. Similar results were found with standards containing known amounts of ATP, where only the standard containing the highest concentration of ATP would occasionally yield light emissions higher than the blank.

ATP From Natural Populations

The plankton of several lakes was sampled during the summers of 1978 and 1979 to provide a further comparison of filtration and pipetting procedures and to determine whether the pipetting method could be applied to natural planktonic populations.

ATP samples collected by filtration were a variable fraction of those collected by pipetting. Preliminary results from 10 lakes indicated that samples collected by filtration contained only 15–70% (mean 39%) of the ATP that was recoverable by pipetting. In two lakes, ATP from filtered samples exceeded that in pipetted samples by up to 20%. In 1979 (Table 5), collection of ATP samples by pipetting was successful over a range of chlorophyll *a* concentrations from 12 to 310 μg·L^{-1}. In all cases for the five lakes sampled, the filtered estimate of ATP was less than half of the amounts found in the pipetted samples. Dissolved ATP was found in the filtrate from the five lakes, but the levels were far too low to make up the difference between the filtered and the pipetted samples, even though some of this soluble ATP may have included ATP released as a result of cell breakage.

Discussion

ATP Extraction Method

Filtration results in a much lower estimate of ATP than pipetting, as reported by Jones and Simon (1977), in both culture and lake samples. This may be due to cell breakage during filtration or ATP hydrolysis during hot extraction before enzyme denaturation is complete. However, if this were true, it is surprising that a larger fraction of the ATP was not recovered in the filtrate and that the use of different filter sizes did not have a consistent effect, as may have been expected if cell breakage during filtration were a major problem. Furthermore, the fact that the use of hot beakers, which should lessen the sharp temperature gradients during heating described by Karl and

LaRock (1975), did not consistently improve extraction argues against major hydrolysis during extraction. While the cause of low ATP recovery from filtration remains unknown, the results suggest that variations in the filtration method are not likely to improve recovery significantly. Perry et al. (1979) reported similar findings to these when comparing amounts of ATP extracted on 47-mm versus 25-mm membrane filters. Higher pipetted ATP levels were not due to soluble ATP because ATP in the filtrates, when detectable, was insufficient to account for the difference between pipetted and filtered samples. Furthermore, in P-deficient cultures, alkaline phosphatase would prevent the accumulation of significant soluble ATP.

The pipetting method proved useable with cultures and denser lake phytoplankton samples (down to 12 μg Chl $a \cdot L^{-1}$). To obtain particulate ATP using this method, one must measure dissolved ATP as well as total ATP. Dissolved ATP has been reported to be a high percentage of total ATP in seawater (Azam and Hodson 1977) and freshwater (Riemann 1979). Although dissolved ATP was a lower percentage in our lake samples, absolute concentrations of dissolved ATP were similar.

Generally growth into P deficiency lowered P/C ratios to values associated with severe P deficiency, whereas growth into N deficiency had no effect on this ratio. The N/C ratio was lowered by growth into both N and P deficiency. Changes in these ratios were comparable with those seen in previous studies on N- or P-deficient algae (Healey and Hendzel 1979; Riemann and Wium-Andersen 1981), and confirm the occurrence of nutrient limitations here.

The use of ATP to estimate living C is dependent on a constant ATP/C ratio in living microbes. This ratio was found to be approximately constant for exponentially growing algae (Holm-Hansen 1970) and bacteria (Hamilton and Holm-Hansen 1967), where the ratio was very close to 3.5 μg ATP$\cdot$mg C^{-1} for algae and in the range 3–10 μg ATP$\cdot$mg C^{-1} for bacteria. Most of our values for exponentially growing cells were lower than these. The ATP content of algae is not affected by light–dark transitions (Holm-Hansen 1970). However, there are many indications in the literature that the ATP/C ratio can be depressed by nutrient deficiencies (Holm-Hansen 1970; Bottomley and Stewart 1976; Cavari 1976; Healey 1979; Riemann and Wium-Andersen 1981). Our results provide further confirmation of this. The ATP/C ratio was depressed by P and to a lesser extent N deficiency, but in a manner variable from one species to another. Furthermore, depression of the ATP/C ratio could not be related to changes in viability and so could not be attributed to accumulation of dead cells.

Karl and Craven (1980) suggested that hydrolysis of ATP by alkaline phosphatase, which may remain active in ATP extracts, may explain some of the loss of ATP in samples from P-deficient cultures. This cannot account for depressed ATP/C in N-deficient algae, where induction of alkaline phosphatase is not expected. Even where high levels of alkaline phosphatase were present in the cultures, it did not seem to be the cause of ATP loss in the extracts, as temperature and the addition of P had little influence on the rate of short-term ATP loss from extracts. Loss of ATP in extracts from P-deficient cultures was not greater than that from P-sufficient cultures, in contrast with what would be expected if alkaline phosphatase were important. Also, long-term stability of ATP in frozen extracts was not affected by the degree of P deficiency and, therefore, the alkaline phosphatase activity of the originating culture.

Addition of P to P-deficient algae has been reported to result in a rapid increase in the ATP/C ratio (Batterton and van Baalen 1968; Stewart and Alexander 1971; Healey 1979), while addition of N to a N-deficient culture had much less immediate effect on ATP/C (Healey 1979). Except for P-deficient *S. quadricauda* and to a lesser extent P-deficient *A. braunii*, we saw no noticeable increase in ATP/C within 1 h of addition of the deficient nutrient. Presumably, recovery of ATP levels takes place more slowly, on a time scale similar to photosynthesis, making the ATP response to nutrient addition not a generally useful indicator of nutrient deficiency. However, we found the chlorophyll/ATP ratio generally to be unchanged or to rise with P deficiency but to drop with N deficiency, as previously seen (Sakshaug and Holm-Hansen 1977). This response may be useful in distinguishing N and P deficiencies in natural populations.

The results reported here and elsewhere put severe restrictions on the interpretation of ATP measurements. Because of the large variations in ATP/C ratios that can be expected as a result of nutrient deficiencies, ATP cannot be accepted without reservations as an index of living C. However, it still may remain useful as an indicator of living biomass where other evidence indicates that nutrient deficiency is not severe, as in systems that appear mainly light limited (Hecky and Guildford 1984). The response of the ATP/C ratio to nutrient deficiency, particularly to P deficiency, can be used to advantage. Where detrital interference is small, the ATP/C ratio may be useful as an indicator of P deficiency (Cavari 1976; Healey and Hendzel 1980). Because ATP/C generally falls while such parameters as P uptake and alkaline phosphatase activity per unit C rise in P deficiency, the ratios of these parameters to ATP can be sensitive indicators of P deficiency (Healey and Hendzel 1979, 1980). Our results and those of Jones and Simon (1977) strongly indicate that collection by filtration underestimates ATP by a variable and often great amount. However, both the reason for this and its generality remain obscure. The fact that early results on exponential cultures (Holm-Hansen 1970) were higher and more uniform than ours shows that filtration does not always lead to low ATP results. Table 1 shows that the filtration error is much more severe for some algae than for others. The potential for this underestimate should be kept in mind when reviewing earlier ATP data, and further comparisons of filtered and pipetted results are needed to establish the severity and generality of this filtration error.

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Phosphate Exchange Between Littoral Sediments and Overlying Water in an Oligotrophic North-Temperate Lake[1]

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The soluble phosphate equilibrium between aerobic sediment and overlying water was examined in intact cores of marginal sediment and water from Lake Memphremagog. Radiotracer experiments demonstrated a dynamic phosphate exchange between sediments and water under steady-state conditions. Perturbations of this equilibrium in batch and continuous-flow systems show that aerobic sediment can act as a phosphate source or sink, depending on the phosphate concentration of the overlying water. The existence of an oxidized microzone on the sediment surface does not preclude phosphate release when concentrations in the overlying water drop below the equilibrium concentration. Under natural conditions, soluble reactive phosphorus concentrations in the surface waters of Lake Memphremagog are always lower than the equilibrium concentrations measured in this study. This suggests that aerobic marginal sediments may serve as a phosphate source in lakes where algal demand maintains low levels of soluble phosphate in the water.

L'équilibre du phosphate soluble entre les sédiments aérobies et l'eau qui les recouvrent a été étudié à partir de carottes intactes de sédiments marginaux et d'eau du lac Memphrémagog. Des expériences réalisées à l'aide de marqueurs radioactifs ont mis en évidence les réactions dynamiques d'échange du phosphate qui se produisent entre les sédiments et l'eau dans des conditions stables. En perturbant cet équilibre dans des systèmes immobiles et à écoulement continu, on voit que les sédiments aérobies peuvent agir comme source ou réceptacle de phosphate, suivant la concentration de phosphate de l'eau. L'existence d'une micro-zone oxydée à la surface des sédiments n'empêche pas le phosphate de s'échapper lorsque la concentration de phosphate dans l'eau tombe en-dessous de la concentration d'équilibre. Dans des conditions naturelles, les concentrations de phosphore soluble réactif dans les eaux superficielles du lac Memphrémagog sont toujours inférieures aux concentrations d'équilibre mesurées dans la présente étude. Il semblerait donc que les sédiments marginaux aérobies servent de source de phosphate dans les lacs où les besoins des algues maintiennent à un niveau faible la concentration du phosphate soluble dans l'eau.

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Although phosphorus cycling in lakes has been extensively studied and sediments have been identified as a major component of the phosphorus cycle (Syers et al. 1973; Bostrom et al. 1982), the role of lake sediments in phosphorus budgets is still not clear. Phosphorus accumulation in the surface sediments of lakes and phosphorus retention in lake nutrient budgets (Bostrom et al. 1982; Håkanson and Jansson 1983) indicate a net flux of phosphorus into lake sediments. However, lake sediments, like terrestrial soils, can exchange orthophosphate (PO_4) with the surrounding water (Li et al. 1972; Logan 1982), and since PO_4 appears to be the primary form of available phosphorus (Lean 1973), this exchange can be important in phosphorus cycling.

The best documented mechanism of PO_4 exchange across the sediment–water interface involves the formation of an Fe–PO_4 complex under aerobic conditions and its dissolution in anoxia (e.g. Mortimer 1941). On the basis of this, aerobic sediments have, until recently, generally been regarded as exclusive phosphate sinks (Håkanson and Jansson 1983). Phosphate release from lake sediments under aerobic conditions has been reported from both eutrophic and oligotrophic waters (Lean and Charlton 1976; Ryding and Forsberg 1976; Ulen 1978; Theis and McCabe 1978; Gallepp 1979; Graneli 1979; Holdren and Armstrong 1980; Bates and Neafus 1980; Twinch and Breen 1981; Andersen 1982; Bostrom and Pettersson 1982; Grizzard et al. 1982; van Liere and Mur 1982).

In general, sediments may serve a dual role as phosphorus source or sink, but the factors that control the directions, rates, and magnitudes of the fluxes of phosphorus between sediments and overlying water have not been clearly identified or quantified. Under aerobic conditions, sediment–water PO_4 exchange probably depends on factors such as mineral–water equilibria, adsorption–desorption processes, mixing patterns in the water, and benthic biological activity (Bostrom et al. 1982). The overall effect is that aerobic lake sediments can approxi-

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mate a dynamic equilibrium with dissolved PO_4 (Gumerman 1970; Li et al. 1972; Ku et al. 1978; Lean and Charlton 1976; Logan 1982).

The direction of PO_4 flux across the sediment–water interface reflects the PO_4 concentration gradient between the sediment pore water and the overlying water (Olsen 1958, 1964; Golterman 1973; Imboden and Lerman 1980; Thies and McCabe 1978). If the PO_4 equilibrium is disturbed by PO_4 enrichment of the overlying water, a flux into the sediment may occur (Bostrum et al. 1982). Conversely, if algal demand in the water reduces PO_4 levels below the equilibrium concentration, sediment release of PO_4 can be induced (Golterman 1973). This equilibrium accounts for the buffering of changes in PO_4 concentration in overlying waters by the sediments (Meyer 1979).

This study set out to assess the role of aerobic marginal sediments in phosphorus cycling in Lake Memphremagog by experimental manipulation of the sediment–water phosphate equilibrium. We wished to quantify the amount of flux between aerobic sediments and the water and to estimate if this flux might affect the phosphorus supply to the plankton.

Materials and Methods

Sediment Sampling and Handling

A single site in Quinn Bay (40°04′N, 72°16′W), midway along the eastern shoreline of Lake Memphremagog (Quebec–Vermont), was used throughout this study. This site was selected for the availability of an extensive area of homogeneous sediment in water approximately 5 m deep just beyond the zone of rooted aquatic macrophytes. All experiments were undertaken on intact sediment cores. To collect samples, SCUBA divers pushed Plexiglas tubes (4.5 or 5.0 cm internal diameter) about 15 cm into the sediment, withdrew intact sediment cores, and sealed the tubes for transport to the laboratory. There, the top seals were removed (to allow gaseous exchange) and the samples were left undisturbed for at least 1 h (to allow setting of resuspended material).

To manipulate PO_4 gradients between sediments and overlying water we employed two types of sediment–water systems: in batch systems, the overlying water remained in contact with the sediment for the duration of the experiment; in continuous-flow systems, a pump continuously replaced the overlying water. To set up batch systems, the overlying water volume was adjusted to between 250 and 350 mL and the phosphorus pool in the water was manipulated by addition of tracer, PO_4-rich, or PO_4-deficient solutions to the water. The water phase was always gently mixed before sampling to ensure homogeneity. Since the overlying water was open to the atmosphere, oxygen concentration in the water was assumed to remain close to saturation. Continuous-flow systems held smaller volumes (15–30 mL) of overlying water to ensure rapid attainment of steady state. A sealed chamber attached to the tubes by means of rubber O-rings provided an inlet and outlet port, and a peristaltic pump provided a continuous flow of aerated water. Overlying water volume was controlled by vertical adjustment of the outlet port to maintain a depth of about 1 cm. The chamber could be replaced periodically to reduce wall effects associated with accumulation of attached organisms. The PO_4 concentration of the inflowing water was controlled, and uptake or release of PO_4 was calculated from differences between soluble reactive phosphorus concentrations in inflows and outflows. Oxygen saturation in the inflows was maintained by continuous aeration of the inflow reservoir.

All sediment samples were collected during the summer months of 1980 and 1981, and three replicates were used for each treatment in all experiments. The experiments were conducted at ambient laboratory temperatures (approximately 20 ± 3°C).

Phosphorus Analysis

Phosphorus fractions in the water were analysed using the single solution molybdate blue procedure (Murphy and Riley 1962). Soluble reactive phosphorus (SRP) was measured on filtered water (0.45 μm pore size); total phosphorus (TP) and total soluble phosphorus (TSP) were measured on whole and filtered water, respectively, after digestion with potassium persulphate at 120°C for 1 h (Menzel and Corwin 1965). Particulate phosphorus (PP) and soluble unreactive phosphorus (SUP) were calculated by difference from TP and TSP (i.e. PP = TP − TSP and SUP = TSP − SRP). Depending on concentration, either 1- or 10-cm cuvettes were used, giving a theoretical minimum detection level of about 0.3 $\mu g \cdot L^{-1}$.

Measurement of Phosphorus Fluxes

The initial experiment was designed to demonstrate and quantify "steady-state" PO_4 exchange between sediments and overlying water. Enough radiotracer (carrier free ^{32}P as PO_4 in dilute HCl, New England Nuclear Corp.) was added to the water phase of three identical batch systems to yield about 10^4 Bq/mL. Partitioning of tracer between water and sediment with time was monitored by withdrawing 1-mL samples and measuring total radioactivity by Cerenkov counting after dilution with 10 mL of distilled water. A control system containing only bottom water sampled at the time of the sediment sampling was monitored in a similar way. At the end of the experiments, particulate and soluble ^{32}P in the water was determined by difference between total radioactivity in filtered and unfiltered (0.45 μm) samples. Changes in the concentrations of phosphorus fractions in the water were measured simultaneously in three unlabelled, but otherwise identical, batch systems.

The rates of PO_4 uptake by sediments were measured in batch systems enriched with varying amounts of PO_4 as KH_2PO_4 solution (three replicates at four concentrations between 0 and 150 $\mu g \cdot L^{-1}$ were used on four separate occasions). Enrichments were applied to the water phase, and changes in the concentrations of phosphorus fractions in the water monitored for 12 h. Initially, PP, SUP, and SRP were monitored but when it became evident that only SRP changed significantly, only that fraction was measured.

Measurement of phosphorus release rates from sediments in batch systems was hampered by resuspension of surface sediments during the replacement of overlying water with phosphorus-deficient algal medium (PAAP) (USEPA 1969). Smooth release curves were not obtained, although after a period of settling, the equilibrium concentrations appeared to be reestablished. Consequently, four experiments were conducted in which water overlying freshly sampled sediment–water systems was removed and replaced with 100 mL of phosphorus-deficient algal medium. After at least 12 h, SRP concentrations in the overlying water were measured to assess whether SRP release had occurred and whether the equilibrium concentration was reestablished.

Unlike batch systems, continuous-flow systems allowed the maintenance of PO_4 gradients across the sediment–water interface for extended periods of time. They also reduced the problem of sediment resuspension in batch systems by facilitating more gentle removal of the original overlying water. In continuous-flow systems, a PO_4 gradient into the sediments was induced by using PO_4-enriched lake water as influent. This was prepared by adding KH_2PO_4 to filtered (0.45 μm) lake water which was sterilized before or after addition of the PO_4. In the former case, some PO_4 loss (about 15%) due to precipitation was evident. The opposite gradient was induced by using phosphorus-deficient algal medium, diluted to half-strength, as influent. Single, week-long experiments using triplicate continuous-flow systems were conducted to monitor PO_4 uptake and release independently. Flow rates during the uptake experiment were maintained at 78 $mL \cdot h^{-1}$ throughout. During the release experiment, SRP concentrations in the outflow were low (usually below 4 $\mu g \cdot L^{-1}$) at this flow rate. After 20 h, the flow rate was slowed to 40 $mL \cdot h^{-1}$ to increase contact time between sediment and water in an unsuccessful attempt to increase SRP concentrations in the outflows.

Results

Radiotracer Experiments

Uptake of ^{32}P in three sediment–water batch systems showed a similar pattern: about 10% of the added tracer remained in the water after 50 h, and an apparent asymptotic level (mean 2%) in the water was reached by 170 h (Fig. 1). In the control, the rate and extent of tracer removal from the water were much lower, indicating that sediment uptake of ^{32}P was the major pathway of tracer loss in sediment–water systems. However, ^{32}P uptake in the control indicates the potential importance of attached microorganisms in all systems, particularly during prolonged experiments: after 12 h, only about 2% of the tracer in the control had been removed but the removal increased to 15% after 50 h and 23% after 170 h. Since the role of attached organisms was negligible during the first 12 h, subsequent uptake experiments in batch systems were limited to 12-h duration during which it was assumed that a maximum of 2% of PO_4 uptake was due to attached organisms in the Plexiglas tubes.

Despite the rapid ^{32}P uptake by sediments, there was no corresponding change in the concentration of phosphorus fractions in the overlying water (Fig. 2). During the first 12 h, PP, SUP, and SRP in the overlying water remained stable. However, by the end of the experiment, both PP and SUP had increased by about 5–10 $\mu g \cdot L^{-1}$. SRP remained stable throughout. In the control, SUP declined from 6 to 2.5 $\mu g \cdot L^{-1}$ during the experiment, while PP and SRP showed little change.

Figures 1 and 2 show that ^{32}P uptake does not reflect net changes in the phosphorus status of the overlying water, but rather tracer dilution into the total exchangeable phosphorus pool (water plus sediments) via a dynamic equilibrium whereby phosphorus influx into the sediments is matched by a corresponding efflux. Under such steady-state conditions, ^{32}P uptake by sediments continues until the exchanging phosphorus is uniformly labelled. At this point, there is no further net uptake of tracer. The ^{32}P uptake data were adequately described as a single exponential approach to an asymptote ($^{32}P - {}^{32}P$ asymptote $= 93e^{-0.05t}$, $r^2 = 0.97$) typical of monophasic

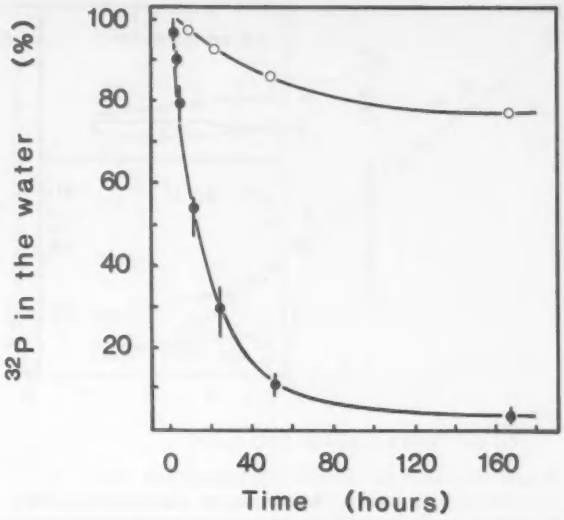

FIG. 1. Uptake of ^{32}P from the water phase of sediment–water batch systems (○) and in a control system containing no sediment (●). Vertical bars indicate ranges for three replicates.

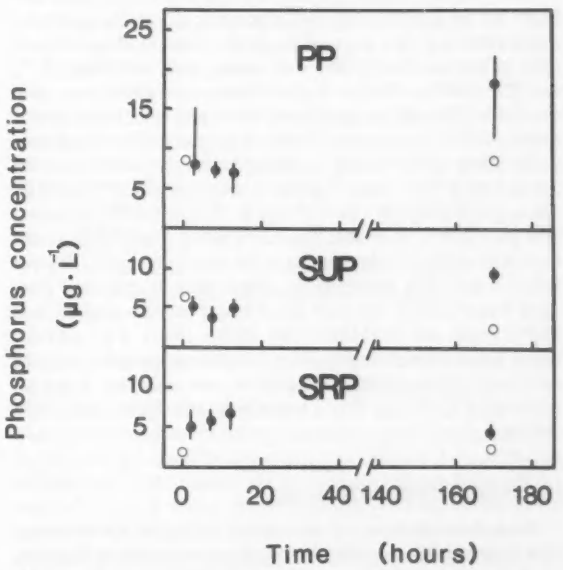

FIG. 2. Concentrations of PP, SUP, and SRP in the water phase of sediment–water batch systems (○) and in a control containing no sediment (●). Vertical bars indicate ranges for three replicates.

exchange kinetics. At isotopic equilibrium, 97% of the tracer was in the sediments, 1.4% in the suspended particulate material, and 0.6% in the soluble fraction (<0.45 μm) in the overlying water. This distribution gives a relative index of the pool sizes of exchangeable phosphorus associated with each fraction in the systems. The standard isotopic dilution equation (Wang et al. 1975) was used to estimate the amount of sediment exchangeable phosphorus:

(1) Sediment ^{31}P/soluble ^{31}P = sediment ^{32}P/soluble ^{32}P.

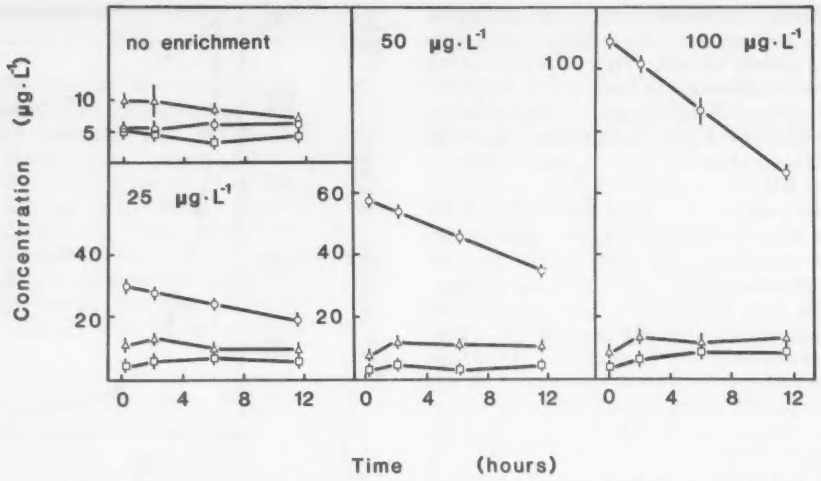

FIG. 3. Changes in the concentration of PP (△), SRP (○), and SUP (□) in the water phase of sediment–water batch systems that received the indicated PO_4-P enrichments. Vertical bars represent 1 SD ($n = 3$).

Two soluble phosphorus fractions were measured (SRP and SUP) but the specific activities of these could not be calculated independently, since we undertook no corresponding fractionation of the soluble ^{32}P. For this reason, both SRP (5 $\mu g \cdot L^{-1}$) and TSP (SRP + SUP) (15 $\mu g \cdot L^{-1}$) concentrations were used as soluble ^{31}P in the calculations. The former calculation gives a lower limit to the amount of sediment exchangeable phosphorus in the event of SUP being unlabelled, and the latter yields the upper limit in the event of uniform labelling of SRP and SUP. These give a range of 114–455 $mg \cdot m^{-2}$ of sediment exchangeable phosphorus in aerobic sediments under steady-state conditions with respect to phosphorus in the overlying water. The true value is probably intermediate, since there is evidence from open water studies that SUP has a lower specific activity than SRP (Levine and Schindler 1980; Peters 1981). This estimate based on sediment surface area is not comparable with the sediment exchangeable phorphorus per unit dry mass of sediment (32–60 $\mu g\ P \cdot g^{-1}$) made by Carignan and Kalff (1979).

Uptake and Release Experiments

Since the radiotracer experiments confirmed the existence of a dynamic PO_4 equilibrium between sediments and aerobic overlying water, the remainder of this study was directed at quantifying the potential rates of PO_4 uptake and release by sediments in response to direct perturbations of the equilibrium. The analysis presumes that SRP is equivalent to PO_4 although this is controversial (Tarapchak et al. 1982a, 1982b). Rigler (1966) estimated that SRP analyses may overestimate PO_4 levels by 10–100 times, but Peters (1979) in Lake Memphremagog and Stainton (1980) elsewhere found the discrepancy to be considerably less. Nürnberg and Peters (1984a) were unable to show a consistent difference between SRP and PO_4 concentrations reported in the literature. This study also provides evidence that changes in SRP are an adequate index of PO_4 flux across the sediment–water interface. SRP is used in this capacity because no convenient alternative exists.

Batch systems

In unenriched systems, the phosphorus status remained virtually stable during the 12-h experiment (Fig. 3): PP showed a negligible decline and concentrations remained at about 10 $\mu g \cdot L^{-1}$; SRP and SUP fluctuated slightly around 5 $\mu g \cdot L^{-1}$. The responses in the unenriched systems were thus similar to responses in the systems used during the radiotracer experiment (Fig. 2). At all levels of PO_4 addition, the SRP concentration declined linearly for the duration of the experiment (Table 1). This decline was not matched by a corresponding increase in PP or SUP (Fig. 3), although both increased from just less than 10 $\mu g \cdot L^{-1}$ to about 15 $\mu g \cdot L^{-1}$ during the first 2 h in both 50- and 100-$\mu g \cdot L^{-1}$ treatments, thereafter remaining stable. To judge from the limited influence of attached microorganisms on ^{32}P uptake during the first 12 h in the tracer experiments (Fig. 1), their influence in these experiments was probably negligible. The SRP uptake from the water must, therefore, reflect direct uptake by the sediments.

In all, 36 triplicate sets of sediment–water batch systems were enriched with sufficient PO_4 to produce a range of initial SRP concentrations of from 12 to 145 $\mu g \cdot L^{-1}$. Unenriched systems were monitored simultaneously, and a summary of phosphorus partitioning in the overlying water in these is presented in Table 2. Mean concentrations of SRP, SUP, and PP in the unenriched systems represented 26, 23, and 51% of the mean total phosphorus content in the water, respectively. Over the range of concentrations used, the increase in uptake rate with increasing concentration could be described by a linear function (Fig. 4). These experiments were undertaken over a period of 3 mo during summer 1980, and the variability of Fig. 4 ($r^2 = 0.81$) probably reflects experimental differences among runs as well as spatial and seasonal changes in the sediments.

Aerobic sediment can serve as a PO_4 source when concentrations in the overlying water are reduced (Table 3). During these experiments the SRP levels, after at least 12 h of equilibration (mean = 5.6 $\mu g \cdot L^{-1}$, SD = 1.5), were similar to those measured in untreated sediment–water systems (mean 4.6 $\mu g \cdot L^{-1}$, SD = 2.4) (Table 2). This indicates that the sediments are able to

TABLE 1. Coefficients obtained by fitting the data in Fig. 4 to the model $Y = a + bt$ by least squares regression (Y *is the concentration of SRP* ($\mu g \cdot L^{-1}$) remaining at time t (h)). The rate is calculated from the slope of the regression and the area of the core. Means for three replicates are shown.

Enrichment ($\mu g \cdot L^{-1}$)	r^2	a ($\mu g \cdot L^{-1}$)	b ($\mu g \cdot L^{-1} \cdot h^{-1}$)	Rate ($\mu g \cdot m^{-2} \cdot h^{-1}$)
25	0.98	29.3	−0.94	119
50	0.99	59.0	−2.06	262
100	0.98	109.7	−3.63	462

TABLE 2. Phosphorus partitioning in the water phase of unenriched sediment–water batch systems. Measurements made within 24 h of collecting the sediment cores.

	SRP	SUP	PP
Mean concentration ($\mu g \cdot L^{-1}$)	4.6	4.0	8.9
Min concentration ($\mu g \cdot L^{-1}$)	1.0	1.7	6.0
Max concentration ($\mu g \cdot L^{-1}$)	11.8	9.0	14.9
Standard deviation	2.4	1.8	2.6
Number of measurements	41	12	12

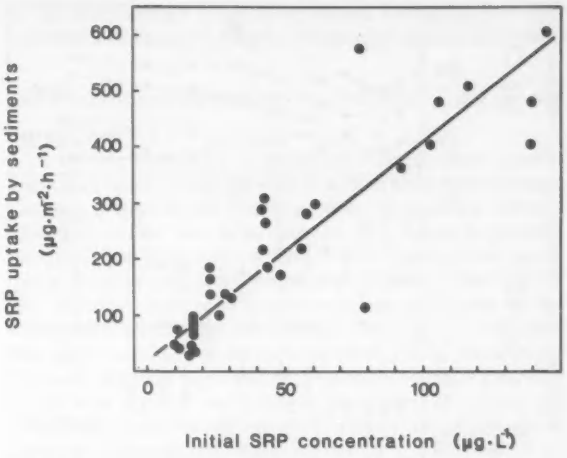

FIG. 4. Scatter plot of initial SRP concentration in the water phase of sediment–water batch systems versus sediment SRP uptake rate. Linear regression equation fitted by least squares analysis: uptake rate = 3.9SRP + 23 ($n = 36$, $r^2 = 0.81$).

reestablish equilibrium SRP concentrations in the water. This is so even after prolonged leaching of PO_4 during the continuous-flow release experiment.

Continuous-flow systems

Uptake of PO_4 by intact cores in continuous-flow systems was monitored for 200 h (Fig. 5). During the experiment, a change in the procedure for sterilizing enriched lake water resulted in a change in the inflowing SRP concentration (Fig. 5A). Up to 80 h, the influent was heat sterilized after receiving enough PO_4 to increase the SRP concentration by 50 $\mu g \cdot L^{-1}$. Some of the added phosphorus was incorporated into PP and/or SUP, since the inflow concentration of SRP was only about 40 $\mu g \cdot L^{-1}$. After 80 h, PO_4 was added to presterilized lake water at room temperature; SRP concentrations increased to the expected level and the PP + SUP showed a corresponding drop. During the first 40 h of the experiment, PP + SUP in the outflows tended to be higher than in the inflows (Fig. 5B). Some of this increase may reflect incorporation of PO_4 by suspended particles, but since the influent water was sterile, much of the increased PP probably consists of phosphorus associated with eroded sediment. Sediment uptake of PO_4 is reflected in the consistently lower SRP concentrations in the effluent. Differences in SRP between inflows and outflows tended to be higher during the first 24 h but then stabilized. There was no discernable response to the change in inflow SRP concentration. Sediment uptake rate (Fig. 5C) was calculated from the observed changes in inflow and outflow SRP concentrations and the flow rate. No evidence of a decline in uptake rate was evident by the end of the experiment, so no limits to the PO_4 uptake potential could be estimated. Initially the rates measured in the continous-flow system tended to be higher than predicted (Fig. 5C), but after about 40 h, the measured and predicted rates showed reasonable agreement.

Release of PO_4 from sediments was monitored in a similar continuous-flow experiment using phosphorus-deficient algal medium as influent (Fig. 6). Since the experiment was aimed at measuring PO_4 release rates, only SRP was measured. The SRP concentration in the inflow medium remained between 1 and 1.8 $\mu g \cdot L^{-1}$ (mean = 1.4, SD = 0.28) throughout the 190-h experiment. SRP concentrations in the outflow varied fairly widely within and amongst replicates (Fig. 6A) but were always higher than the mean inflow concentration ($n = 51$). Although differences in SRP concentration between inflows and outflows were often small during this experiment (on two occasions the lower limit of the outflow range overlapped with the upper 95% confidence limit for the inflow concentration), they differed significantly overall ($P = 0.001$, normal approximation of the Mann–Whitney test, Zar 1974). During the first 19 h (flow rate = 78 $mL \cdot h^{-1}$), the outflow concentration varied between 1.6 and 6.6 $\mu g \cdot L^{-1}$, and during this period the mean difference between inflow and outflow concentrations was 1.7 $\mu g \cdot L^{-1}$ (SD = 1.16). Once flow rate was reduced to 40 $mL \cdot h^{-1}$, the outflow concentrations showed less variability and the mean difference between inflows and outflows declined to 1.01 $\mu g \cdot L^{-1}$ (SD = 0.22). Since no decline in release rate was evident after 240 h, no assessment of the extent of the potentially exchangeable phosphorus pool in the sediments could be made from these data. During the period of faster flow, the mean release rate was 85 $\mu g \cdot m^{-2} \cdot h^{-1}$ (SD = 56) and during the period of slower flow was 25 $\mu g \cdot m^{-2} \cdot h^{-1}$ (SD = 5).

TABLE 3. Change in SRP concentration in phosphorus-deficient water after at least 12 h of contact time with intact sediment cores (UD = undetectable; standard deviation ($n = 3$) in parentheses).

Date	Initial SRP ($\mu g \cdot L^{-1}$)	Final SRP ($\mu g \cdot L^{-1}$)
June 4, 1980	1.8	5.1 (0.72)
October 21, 1980	1.3	6.5 (1.00)
October 22, 1980[a]	1.2	6.4 (0.40)
July 21, 1981	UD	4.5 (2.37)

[a]Measured after continuous-flow release experiment.

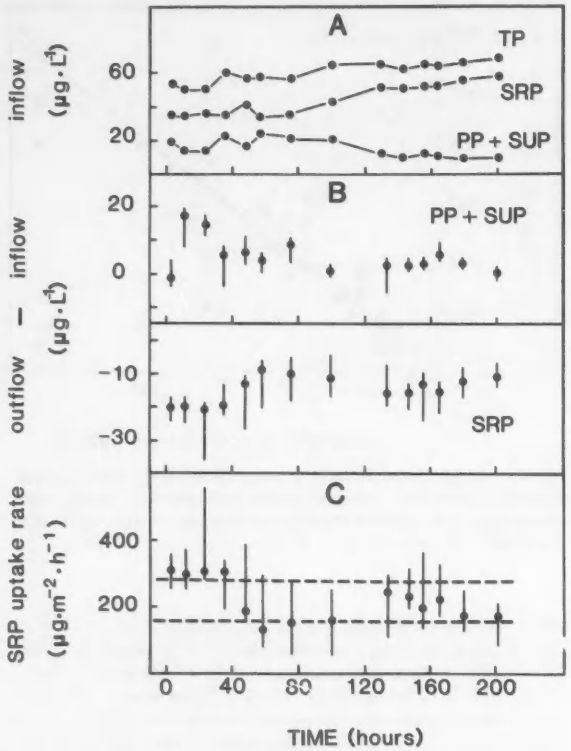

FIG. 5. (A) Concentrations of phosphorus fractions in PO_4-enriched lake water entering continuous-flow sediment–water systems. (B) Differences in PP + SUP and SRP between outflows and inflows. (C) Rates of SRP (PO_4) uptake by sediments. Broken lines indicate upper and lower limits of uptake rates predicted from inflow concentration using the regression equation in Fig. 5. Mean values plotted; vertical bars indicate ranges for three replicates.

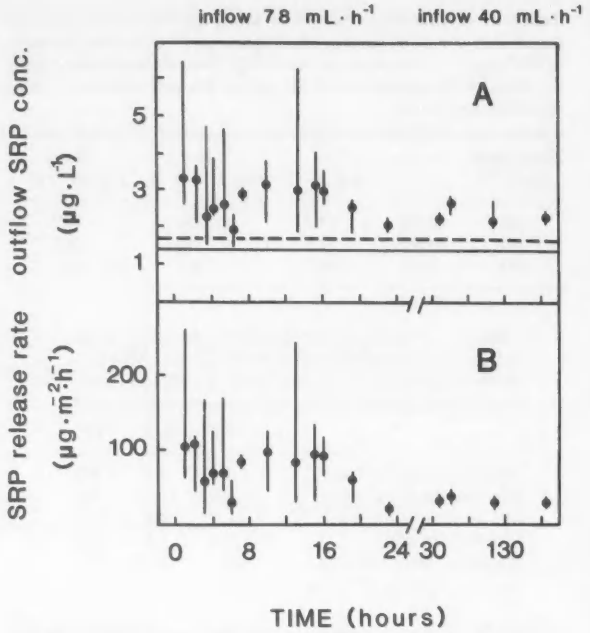

FIG. 6. (A) SRP concentrations in outflows from continuous flow sediment–water systems eluted with phosphorus-deficient water. Solid and broken lines indicate, respectively, the mean and upper confidence limit ($P = 0.05$) for inflow SRP concentration ($n = 20$). (B) Rates of SRP (PO_4) release from sediments. Mean values plotted; vertical bars indicate ranges for three replicates.

Discussion

Dynamic Equilibrium

The results of these experiments demonstrate the existence of a dynamic PO_4 equilibrium between aerobic littoral sediments and the overlying water. This was most clearly shown by the rapid dilution of added ^{32}P into the sediment phosphorus pool without changes in the phosphorus status of the overlying water. The dynamic steady-state PO_4 exchange between sediments and water (Li et al. 1972) allows the sediments to buffer changes in PO_4 concentration of lake waters (Hayes and Phillips 1958; Gumerman 1970; Golterman 1973; Meyer 1979; Logan 1982; and others). Such an equilibrium implies that aerobic sediments may act as a PO_4 source or sink with the direction and rate of flux being controlled by the direction and extent of the PO_4 diffusion gradient between the sediment pore water and the overlying water (Imboden and Lerman 1980; Theis and McCabe 1978).

The monophasic ^{32}P exchange kinetics between sediment cores and overlying water observed during this study contrast with reports of diphasic, or even polyphasic, kinetics in similar experiments (Li et al. 1972; Howard-Williams and Allanson 1978; Twinch and Breen 1982). Li et al. (1972) attributed polyphasic kinetics to the combined effects of several exchange reactions occurring at different rates, but stressed that graphical resolution of ^{32}P exchange curves into distinct phases does not constitute proof of an equivalent number of exchange reactions, particularly if different reactions have similar rates. Thus, in our study, the monophasic ^{32}P uptake data do not imply a single exchange reaction. The data might well imply differences in the relative importance of specific reactions in different sediment–water systems.

Based on the ^{32}P distribution at isotopic equilibrium in batch systems, it was estimated that the pool size of exchangeable phosphorus in the aerobic sediment is between 114 and 455 $mg \cdot m^{-2}$. During the continuous-flow experiment, PO_4 was released from the sediments at a minimum rate of 25 $\mu g \cdot m^{-2} \cdot h^{-1}$ for 190 h, resulting in a total sediment phosphorus loss of about 5 $mg \cdot m^{-2}$ during the experiment. This represents between 1.1 and 4.4% of the estimated total exchangeable pool, which suggests that the measured release rates could be sustained for much longer periods.

Phosphate Uptake and Release

The ability of most sediments to release some PO_4 under aerobic conditions has been indisputably demonstrated using a bioassay approach in which phosphorus associated with suspended sediments is the only form of the nutrient provided to algae in culture (Golterman et al. 1969; Golterman 1973; Fitzgerald 1970; Young and De Pinto 1982; Klapwijk et al. 1982). Whilst these are not analogous to experiments on intact sediment–water systems, because sediments in suspension are in closer contact with the epilimnetic water than is an intact sediment surface, they do demonstrate the bioavailability

of a proportion of sediment phosphorus under aerobic conditions. In principle, therefore, similar release would be expected from the oxidized microzone of aerobic bottom sediments in response to appropriate perturbation of the sediment–water PO_4 equilibrium.

Our study demonstrates the ability of aerobic sediments to reestablish a SRP equilibrium concentration of about 5 $\mu g \cdot L^{-1}$ in the overlying water, following replacement of the original water with phosphorus-deficient water. In addition, sustained SRP release rates of at least 25 $\mu g \cdot m^{-2} \cdot h^{-1}$ for at least 8 d were measured in continuous-flow systems. Aerobic PO_4 release rates measured by various methods from other lake sediments vary widely, from negative values to over 47 $\mu g \cdot m^{-2} \cdot h^{-1}$ (Banoub 1975; Ryding and Forsberg 1976; Reiman 1977; Gallepp 1979; Bostrom et al. 1982; Nürnberg 1984). The wide range reflects the combined influences of varying lake trophic status and varying methods of estimating rates. Because of the importance of internal loading in eutrophication management, the majority of aerobic release rate estimates have been made at eutrophic sites, with little attention to oligotrophic sites. Kamp Nielsen (1974) measured rates (8.3 $\mu g \cdot m^{-2} \cdot h^{-1}$) at oligotrophic Lake Gribs that are slower than those measured in our study, but the methods were different and direct comparisons are tenuous. Expectedly, our aerobic release rates are much slower than release rates measured from anaerobic sediments in Lake Memphremagog (415–541 $\mu g \cdot m^{-2} \cdot h^{-1}$, Nürnberg 1984).

In some instances where zero or negative aerobic phosphate release from lake sediments has been measured (e.g. Viner 1975; Fillos 1976; Kamp Nielsen 1974) it is possible that the sediment–water equilibrium was not suitably manipulated to induce release. If the phosphate concentration applied to the sediments approximates, or exceeds, the equilibrium concentration, zero or negative release rates can be expected. Phosphate release from sediments requires that the phosphate concentration applied be lower than the equilibrium concentration, and in oligotrophic lakes where equilibrium concentrations are lower (e.g. 4.6 $\mu g \cdot L^{-1}$ in our study), careful experimental manipulation is required to quantify release rates.

Apparent aerobic phosphate release from some sediments has been attributed to the possible development of localized anaerobic conditions at the sediment–water interface (Lean and Charlton 1976; Holdren and Armstrong 1980). This could not apply to our continuous-flow experiments used to measure release rates because oxygen saturation in the inflows was maintained and water residence time in the systems was short (about 30 min maximum).

The ability of aerobic sediments to remove PO_4 from solution is well documented (Fitzgerald 1970; Syers et al. 1973; Bostrom et al. 1982) and was confirmed for Quinn Bay sediments during this study. Over the range of PO_4 concentrations used in the rate measurements, the rate of uptake was linearly related to the concentration in the overlying water, as has been shown in other sediments (Theis and McCabe 1978; Twinch 1980). At comparable SRP concentrations in the overlying water, uptake rates by intact cores have varied: Theis and McCabe (1978) measured rates in sediments from two eutrophic lakes that were less than half the rates measured here, Fillos and Biswas (1976) measured similar rates in eutrophic Lake Mohegan, and Twinch (1980) measured rates in an oligotrophic impoundment that were more than double those we measured during our study. In Quinn Bay, the PO_4 uptake ability of the sediments is of little consequence in the phosphorus cycle, since SRP concentrations in the water (Peters 1979) never exceed the equilibrium SRP concentration (mean 4.6 $\mu g \cdot L^{-1}$) measured during this study.

Aerobic Phosphate Release and the Phosphorus Cycle of Lake Memphremagog

A reliable knowledge of phosphate concentrations in sediment pore water is essential when assessing phosphate exchange between sediments and overlying water because the rate and direction of flux are dependent on the phosphate gradient between pore water and overlying water. Furthermore, phosphate diffusion calculations require an estimate of the depth of the sediment layer which can contribute phosphate to the overlying water (Bostrom et al. 1982). Under aerobic conditions the appropriate pore water concentration and the depth of the "active" sediment layer cannot be reliably measured because of the thin oxidized surface layer (sometimes of microscopic depth) that covers the reduced sediments and controls phosphate flux across the sediment–water interface (e.g. Mortimer 1941).

Estimates of phosphate concentration in surface pore water in Lake Memphremagog, using a compartmentalized dialysis sampler, varied between almost 0 and 15 $\mu g \cdot L^{-1}$ at a deep and shallow site, respectively (Carignan and Flett 1981). We did not attempt direct measurement of phosphate concentrations in pore water and assume that equilibrium SRP concentrations measured in our experiments (mean 4.6 $\mu g \cdot L^{-1}$) reflect pore water phosphate concentrations in the aerobic surface sediments. Implicit in this assumption is that, if phosphate concentrations in the overlying water are lower than 4.6 $\mu g \cdot L^{-1}$, the resultant phosphate gradient between pore water and lake water will induce phosphate release from the sediments. Since phosphate concentrations in the lake water in the vicinity of the sediment sampling site are usually close to 0 and never exceed 2.5 $\mu g \cdot L^{-1}$ (Peters 1979), we conclude that, under natural conditions, the direction of phosphate flux is from sediments to water at our sampling site.

The rate of exchange of soluble constituents between sediments and water under nonturbulent conditions depends largely on diffusion (e.g. Hesslein 1980). However, flux rates increase with turbulence in the overlying water and turbulence is the dominant factor controlling the rate of phosphate exchange in mixed systems (Holdren and Armstrong 1980; Bostrom et al. 1982). Shallow waters overlying oxidized sediments are, at least periodically, well mixed and the simple diffusion model is not applicable for rate calculations under these conditions. Under natural conditions, turbulence at the sediment–water interface in shallow water varies widely from virtually nonturbulent conditions during periods of calm to turbulent resuspension during storm events. Furthermore, wind-induced turbulence at the sediment–water interface diminishes with increasing water depth (Håkanson and Jansson 1983), so under constant environmental conditions, turbulence at the sediment surface varies markedly with depth. These variations cannot be simulated in laboratory experiments (Bostrom et al. 1982). Our release rate estimates were made in continuous-flow systems in which well-mixed overlying water was maintained by rapid flushing (water residence time <30 min), and we assume that this represents an intermediate level of turbulence in the marginal zone because resuspension of surface sediments did not occur.

Since SRP concentrations in the lake water (<2.5 $\mu g \cdot L^{-1}$) and in the overlying water in the systems used to measure phosphate release (mean 2 $\mu g \cdot L^{-1}$) were similar, we feel that

our aerobic phosphate release rate (mean 85 $\mu g \cdot m^{-2} \cdot h^{-1}$) is a reasonable estimate of the average rate that can be expected in the marginal zone of the lake.

In lakes, the sediment characteristics can show considerable horizontal variation due to "sediment focusing" (Evans and Rigler 1983), morphometric features, and loading patterns (Bostrom et al. 1982). We did not examine this but a general survey of sediments in Lake Memphremagog has been undertaken (R. Flett, Flett Research Ltd., 9-30 Baylor Avenue, Winnipeg, Man., pers. comm.). Eight sites were examined in water up to 10 m deep. In the top 1 cm of sediment, mean values for loss on ignition (percent), total phosphorus (milligrams per gram), and water content (percent), with coefficients of variation in parentheses, were 14.2 (25), 1.8 (26), and 84 (7), respectively. Loss on ignition and water content of the top 1 cm of sediments used in our study were 20 and 82%, respectively (A. J. Twinch, unpubl. data), and the sediments contained obvious signs of terrestrial plant remains, such as bark, which may explain the higher loss on ignition value than those obtained by Flett. No traces of hydrogen sulphide were evident when working with the sediments, although this was not confirmed analytically. Because of the paucity of data it is difficult to assess how representative our experiments are of phosphate exchange characteristics of marginal sediments in the lake. The use of our phosphate release rates in discussing the potential role of marginal sediments in phosphorus cycling in the lake is therefore intended to provide rough estimates only.

If the mean phosphate release rate measured from aerobic sediment (85 $\mu g \cdot m^{-2} \cdot h^{-1}$) is extrapolated to 15% of the total surface area of the lake (13.5 km^2), an annual release of 10 t of phosphorus can be calculated. Over the entire lake surface area this results in a potential internal loading rate of 112 $mg \cdot m^{-2} \cdot yr^{-1}$, which represents 35% of the external phosphorus loading to the lake (320 $mg \cdot m^{-2} \cdot yr^{-1}$) measured by Carlson et al. (1979).

Phosphorus retention in Lake Memphremagog, calculated by difference from annual phosphorus loading (29 t) and losses through the outflow (8 t) (Carlson et al. 1979), was about 21 t. Daily phosphorus loss by sedimentation from the epilimnia of lakes ranges between 1 and 4.5% of the total phosphorus (Rigler 1973), and a value of 2% has been suggested for Lake Memphremagog (Lazerte, cited in Peters 1979). Using the latter loss rate, assuming an average epilimnion depth of 5 m over the whole lake and a total phosphorus content of 15 $\mu g \cdot L^{-1}$ (Peters 1979), an annual of 49 t (544 $mg \cdot m^{-2} \cdot yr^{-1}$) of phosphorus (or a summer loss of about 25 t, since we do not know if the 2% loss rate applies during the winter) can be calculated. The calculated loss of phosphorus to the sediments exceeds the measured net retention by at least 4 t and possibly 28 t. This suggests that internal recycling of phosphorus between sediments and lake water provides an important input of phosphorus into the epilimnion. Such recycling can take place via particulate material by turbulent resuspension of sediments (Bostrom et al. 1982) or via soluble phosphate, either by transport of anaerobic hypolimnetic phosphorus to the epilimnion in deep stratified waters (Nürnberg 1984; Nürnberg and Peters 1984b) or directly from aerobic sediments as shown in our study. The relative importance of these pathways in accounting for the apparent imbalance between phosphorus retention and phosphorus sedimentation (4–28 t) in Lake Memphremagog is not known but we estimate that between 5 t (based on summer release only) and 10 t could be accounted for by release from aerobic marginal sediments.

These results suggest that aerobic phosphate release from marginal sediments may be an important source of phosphate to plankton in the shallow zones of oligotrophic lakes where a combination of algal demand and well-mixed water maintains low ambient phosphate concentrations in water directly overlying the sediments. Under such conditions the sediment–water phosphate gradient can favour slow but steady flux into the overlying water. In contrast with anaerobic phosphate release, which is clearly evident in the build up of phosphate concentrations in anaerobic hypolimnia, phosphate release from aerobic sediments is usually not detectable from field monitoring of epilimnetic phosphate concentrations because of the low concentrations and well-mixed conditions. However, over extended periods the extent of aerobic release can be similar to, or greater than, anaerobic release (Bostrom et al. 1982). Our study has shown that aerobic sediments in oligotrophic lakes may be more important phosphate sources than has previously been recognized and has stressed the need to consider the dynamic phosphate equilibrium between aerobic sediments and water when attempting to assess the role of sediments in lake phosphorus budgets.

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Importance to Mussels of the Benthic Boundary Layer

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We examined whether populations of the blue mussel *Mytilus edulis* (Linnaeus, 1758) and horse mussel *Modiolus modiolus* (Linnaeus, 1758) could, by their feeding activities, deplete the sestonic food supply. The experiments were conducted in a 5-m-long by 50-cm-wide flume in which a turbulent boundary layer flow was created. The seston concentration was measured both as ATP content and numbers of bacteria by acridine orange staining technique on separate subsamples of seawater. Sampling locations were at the flume inlet and at a point 3.6 m downstream (outlet). Four of the five replicated experiments with blue mussels had significant ATP–seston depletion but no reduction in bacterial numbers at the outlet sampling location. Of three replicated experiments with horse mussels, two had significant ATP–seston depletion and all three had significantly fewer bacterial numbers at the outlet sampling location. We consider that these results provide direct evidence in support of the hypothesis that seawater hydrodynamic factors may be critical in determining the food supply available to suspension-feeding animals. Our results confirm circumstantial evidence on this point obtained from field observations by previous investigators. Experimental verification of seston depletion is a necessary, but not sufficient, step in establishing that suspension-feeding animals may be food limited as required by the trophic group mutual exclusion hypothesis.

Nous avons tenté de déterminer si les populations de moules bleues, *Mytilus edulis* (Linnaeus, 1758), et de modioles, *Modiolus modiolus* (Linnaeus, 1758), pouvaient, en se nourrissant, épuiser la réserve de seston. Les expériences ont été réalisées dans une enceinte de 5 m de long et 50 cm de large dans lequel on a créé un courant turbulent de couche limitrophe. La concentration du seston dans différents échantillons d'eau de mer a été évaluée en mesurant la teneur en ATP et le nombre de bactérie au moyen d'une coloration à l'orangé d'acridine. Les échantillons ont été prélevés à l'entrée de l'enceinte et à un point situé à 3,6 m en aval (sortie). Dans quatre des cinq essais réalisés avec les moules bleues, la teneur du seston en ATP au point de prélèvement situé en aval avait sérieusement diminué, contrairement au nombre de bactéries qui était resté le même. Dans deux des trois essais réalisés avec les modioles, on a obtenu une teneur en ATP nettement diminuée, et dans les trois cas, le nombre de bactéries à la sortie de l'enceinte était beaucoup plus faible. Nous considérons que ces résultats appuient directement l'hypothèse selon laquelle la réserve de nourriture accessible aux animaux se nourrissant d'éléments en suspension est déterminée de façon critique par des facteurs hydrodynamiques de l'eau de mer. Nos résultats confirment les suppositions circonstanciées formulées antérieurement à partir d'observations in situ par d'autres chercheurs. La vérification expérimentale est une condition nécessaire, mais non suffisante, pour déterminer si les réserves de nourriture des animaux qui se nourrissant d'éléments en suspension sont limitées suivant le principe de l'antagonisme trophique.

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In his trophic group mutual exclusion hypothesis, Wildish (1977) postulated that both major macrobenthic trophic groups, deposit ingesters and suspension feeders, are food limited and that an important limiting factor is tidal current velocity immediately above the sediment–water interface. Under some hydrodynamic conditions, as for example, with low current speeds above a mussel bed, the mussels remove food particles (= seston) more rapidly than the process of turbulent transfer can supply seston to the mussels. In this situation, the seston concentration is reduced near the sediment–water interface.

A mathematical model developed by Wildish and Kristmanson (1979) can be used to investigate quantitatively the relationship between growth of suspension feeders, bottom roughness, and tidal current velocity. The rate of turbulent supply of seston to suspension feeders that is dependent on tidal current velocity is correlated with production of suspension-feeding animals (Wildish and Peer 1983), thus providing circumstantial evidence supporting the food-limitation hypothesis. Seston depletion has been reported following field experiments or observations by Glynn (1973), Buss and Jackson (1981), and Wright et al. (1982). In their investigations, it was not possible to control current speed or to observe benthic boundary layer conditions, and consequently, their results are also regarded as circumstantial evidence supportive of the food-limitation hypothesis.

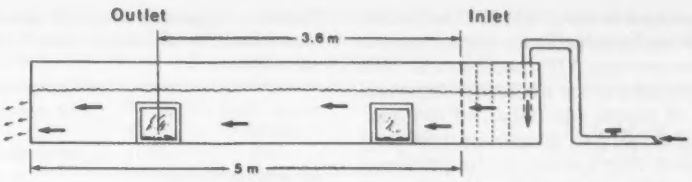

FIG. 1. Laboratory flume used for the seston depletion experiments.

Our work was designed as a direct experimental test to determine whether populations of actively pumping species of suspension feeders were able to deplete seston in a boundary layer as required by the model (Wildish and Kristmanson 1979). A laboratory scale flume was designed and built with the object of generating a boundary layer flow over a bed of mussels. Although this flow field is not identical to the benthic boundary layer found in the sea because of its limited capacity for vertical mixing, it is approximately similar and allows an accurate analysis of the seston balance in it.

Materials and Methods

The model of limited mixing proposed by Wildish and Kristmanson (1979) relies on hydrodynamic arguments presented in the earlier paper. These arguments have been modified to describe the experimental conditions of the work reported here and details may be found in the appendix to this paper.

Experimental Flume

The flume was constructed of plywood supported by wooden beams and metal rods to hold the sides of the flume together (Fig. 1). The total length was 5 m and width of the flume was 50 cm with a flare of a few centimetres from inlet to outlet to ensure an even flow throughout. Seawater entered a mixing chamber provided with three screens in parallel to remove large particles and to help produce an even flow throughout the water column. Seawater left the flume through the back wall, which was provided with numerous holes and rubber bungs so that outlet flow could be controlled. Two Plexiglas windows flush to the inside surface of the plywood wall (Fig. 1) near each end of the apparatus permitted observations to be made.

The flume was set up under a shelter at the top of the shore in front of the Biological Station and was provided with electrical power. Seawater was pumped in a 5-cm plastic line by a 4-hp submsersible pump located 1 m above the sediment near the Biological Station wharf. Seawater from the flume within the boundary layer was sampled with a peristaltic pump with thin plastic tubing (1.5-mm inner diameter) supported on a meter stick with the tip of the tube within 1 cm of the flume bottom. The sampling rate was 110 mL/h and the seawater sample was collected in an autoclaved glass D.O. bottle of 300-mL capacity. With the pump and sampling tube used, isokinetic sampling was achieved at a flume water velocity of ~4 cm/s. Sampling locations were immediately behind the screen (inlet) and at a point 3.6 m downstream from this (outlet) in the center line of the flume.

Experimental Animals

Blue mussels, *Mytilus edulis* (Linnaeus 1758), were obtained from the rocky, exposed shore at Indian Point, St. Andrews. Horse mussels, *Modiolus modiolus* (Linnaeus 1758), were obtained by scuba diving in an area having tidal currents in excess of 100 cm/s at some stages of the tide. The mussels were maintained in large tanks supplied with running seawater from the Biological Station supply.

In setting up the mussel bed, a period of 10 d was allowed for the mussels to acclimate and form byssus attachments. The seawater sampling pump was switched on daily in the morning and the experimental period of 2–4 h begun in the afternoon when at least 95% of the mussels were seen to be gaping and actively pumping. A total of eight experiments with the same population of blue mussels was completed during September and October 1981.

Flow Visualization and Calculation of Flow Velocity

A hydrogen bubble probe (Schraub et al. 1965) was used to investigate flow characteristics in the flume. An electronic black box delivered a square wave of adjustable frequency and pulse width to an electrode pair consisting of a 0.01-cm-diameter platinum wire and a battery-cable ground. Water velocity was measured by finding the pulse width of known time giving a hydrogen bubble sheet that would just fill the gap between the electrode and a reference wire 3.8 cm downstream. Water velocities were calculated from the time taken for a hydrogen bubble to travel the 3.8-cm distance. Use of the finest platinum wire available ensured production of small bubbles with small rise velocities. Because of the delicacy of the thin platinum wires and because in the turbulent layer near the mussels the hydrogen bubble stream moved irregularly and was thus difficult to tune, the hydrogen bubble technique was not suitable for routine use.

Most of the volumetric flow measurements were made in the flume with the aid of a simple orifice meter and water manometer (Anonymous 1971). Hydrogen bubble probe and orifice meter results were in general agreement.

Seston Measurement and Analysis

Processes of addition to the turbulent flume layer, for example, by resuspension of benthic particles from the mussel bed, have been ignored in calculating seston concentration because, in ideal suspension-feeding habitats, both deposition and resuspension are minimal (Wildish and Peer 1983). However, it is obvious that such an omission is not justified in many natural habitats.

Two measures of particulate seston were made. One, using the ATP method, provided a measure of all living matter in the sample inclusive of bacteria, algal cells, and any small animals present. The second was a direct count of bacterial cells by epifluorescence microscopy, as described by Ramsay (1978).

For ATP determinations, 15-mL aliquots of seawater were removed from the sample bottles in a sterile syringe and filtered through an autoclaved Swinnex filter unit containing a 0.45 μm Millipore filter. The filter was removed and boiled for ~30 s in 5 mL of Tris buffer (pH 7.8). After cooling and volume

measurement, the ATP extract was stored at −20°C in the dark until analysis. A purified luciferin–luciferase preparation was used to estimate unknown seawater ATP concentrations from standard ATP solutions prepared in the laboratory. Analytical conditions were 100 μL of sample and 100 μL of luciferin–luciferase in MOPS buffer (pH 7.6) at ambient laboratory temperature. Light flash production was measured in a Biolumat photometer with integration of counts for the first 10 s following automatic injection. Three subsamples were taken from each sample bottle, and three to five replicate ATP determinations were made on each subsample. The typical reproducibility of a net count for ATP determinations from the same unknown extract is 51 253 ± 952 counts/10 s (mean ± standard deviation), $n = 15$, equivalent to 9.2 pg ATP/100-μL sample (range 7.2–10.8). Considerably more variation is introduced when subsamples are drawn from the same bottle (Table 1). This is presumably due to seston "patchiness" in the sample bottle even after vigorous stirring and gives an overall mean = 2.13 ± 0.92 ng ATP/L.

Bacterial counts were made on 1- to 3-mL subsamples drawn from the sample bottle in a sterile syringe and then stored in 10 mL of particle-free, 5% formalin in seawater. This sample was filtered on a 0.22-μm Nucleopore filter stained with Irgulan black. Freshly prepared acridine orange (30 μg/mL) was added to the filter, which was mounted wet on a slide, and then a cover slip and drop of immersion oil were added. The preparation was viewed in a Zeiss Universal microscope set up for epifluorescence with a Zeiss filter combination No. 48 77 09 in place. The excitation filter of this system passed wavelengths in the range 450–490 nm to the chromatic beam splitter, which reflected only wavelengths below 510 nm to illuminate the specimen. The barrier filter allowed light of greater than 520 nm to reach the observer's eye. A Planchromatic 100/1.25 oil immersion objective was used to view the bacteria. Mostly rods and cocci were present and they appeared red or green in color. Counts were made with the aid of an ocular grid (5 × 50 μm); 10 grids were counted so that always greater than 150 bacteria per slide were seen and the mean, standard deviation, and standard error calculated. Typical variation in a sample is shown in Table 2. To determine the number of slides required for an unbiased estimated of the numbers of bacteria present, 10 slides were prepared from the same seawater sample and 10 counts made on each.

Analysis of variance at a 95% confidence interval was used to determine whether mean ATP and bacterial counts were different between different sampling locations in the flume. Seston depletion in the flume was expressed as

$$100C = 100 - \left(\frac{\text{outlet}_{1\,\text{cm}}}{\text{inlet}_{1\,\text{cm}}} \times 100\right)$$

where C is as defined in equation (2) of the appendix. Outlet and inlet samples were taken from 1 cm above the mussels in the flume.

Results

Flow Conditions in the Flume

Measurements were made with the hydrogen bubble generator and blue mussels present in the flume on one occasion between the third and fourth runs of this series of experiments. The results (Fig. 2) show that the flume boundary layer is approximately 10 cm thick at a point 100 cm downstream of the inlet screen and 18–20 cm thick at the outlet sampling station (360 cm from the inlet). Cutting the bulk flow by one half had little effect on the thickness of the flume boundary layer. Development of the boundary layer in the flume led to acceleration of the undisturbed flow from 4.3 to 5.2 cm/s at maximum flow and from 2.7 to 2.9 cm/s at half-flow conditions, which did not have a significant effect on the mass transfer process.

TABLE 1. Concentration of ATP(ng ATP/L seawater) in subsamples drawn from the same seawater sample and replicated analyses on the subsamples.

	ATP replicate determinations from the same extract			
Seawater subsamples	1	2	3	4
1	2.72	3.90	3.36	—
2	2.76	2.58	2.28	—
3	0.60	0.61	0.63	—
4	1.96	2.12	2.09	2.13
5	2.05	2.16	2.19	—

TABLE 2. Numbers of bacteria in replicated samples drawn from seawater ($N = 10$).

Seawater subsample	Mean cells/grid (+ SD)	Mean no. bacteria (millions/mL)
1	18.3±2.2	2.94
2	15.5±2.9	2.49
3	24.6±4.0	2.68
4	19.7±2.7	2.11
5	22.6±4.3	2.42
6	15.9±2.7	1.71
7	19.3±4.9	2.07
8	17.5±5.8	1.88
9	22.6±4.2	2.42
10	16.9±5.2	1.81
Overall	19.4±3.3	2.18±0.34

Seston Depletion by Blue Mussels

The results of repeated subsampling (Table 2) show that reliability of the estimates is increased only slightly. Based on these observations, the subsamples drawn from each sample bottle were reduced to three and the calculated bacterial

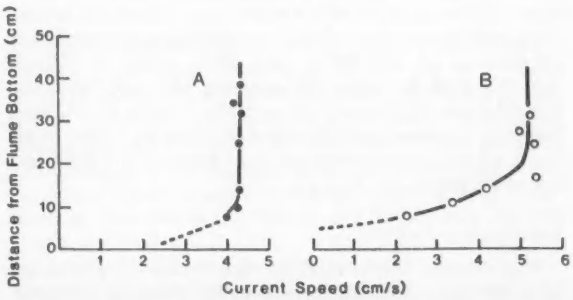

FIG. 2. Typical velocity profiles as measured by the hydrogen bubble probe at (A) 100 cm and (B) 360 cm from the inlet screen.

TABLE 3. Seston concentration above a blue mussel bed of 2123 mussels/m^2 and total wet biomass of 4.848 kg/m^2. Bulk flow velocity was 4–4.5 cm/s. Water sampling was at the outlet end only. N = 9–30; ND = no difference; *$p < 0.05$.

Experiment	Mean temp. (°C)	Height above bottom (cm)	ATP–seston (ng/L), $\bar{X} \pm$ SE		Bacterial nos., $\bar{X} \times 10^6$/mL (±SE)	
1	12.9	30	33.02±2.76	*	4.57±0.70	ND
		1	19.12±4.69		3.79±0.90	
2	13.0	30	27.02±2.82	*	5.28±0.76	ND
		1	21.40±1.85		3.77±0.72	
3	12.6	30	19.15±3.08	*	7.97±0.54	*
		10	13.51±1.15		4.63±0.48	
		5	12.38±0.66	ND	4.38±0.59	ND
		1	13.68±1.58		4.37±1.87	

TABLE 4. Seston depletion over a blue mussel bed 3.6 m long and 0.3 m wide. Mean wet weight of each mussel was 2.283 g. Mussel density was 2123/m^2 in experiments 4–6 and 1062/m^2 in experiments 7 and 8. ND = no difference; *$p < 0.05$.

Experiment	Mean current speed (cm/s)	Mean temp. (°C)	Sample position	ATP–seston $\bar{X} \pm$ SE (ng/L)	ATP–seston % depletion based on inlet 1		Bacterial nos. $\bar{X} \times 10^6$ (±SE)	Bacterial nos. % depletion based on inlet 1	
4	4.10	13.6	Inlet 1	31.86±1.14	0		5.36±0.85	0	
			Outlet 30	29.84±3.22		*	4.33±0.79		ND
			Outlet 1	13.50±0.48	−58		5.72±0.73	+7	
5	4.47	12.8	Inlet 1	25.13±0.55	0		8.54±0.63	0	
			Outlet 30	12.84±1.50		*	5.88±0.79		ND
			Outlet 1	13.53±0.41	−46		5.50±2.89	−36	
6	3.20	12.7	Inlet 1	47.68±3.27	0		9.16±2.47	0	
			Outlet 30	20.27±0.71		*	5.95±2.21		ND
			Outlet 1	8.96±0.95	−81		7.01±2.19	−23	
7	4.18	12.2	Inlet 1	6.92±0.28	0		7.52±1.88	0	
			Outlet 30	5.77±0.08		ND	7.19±3.03		ND
			Outlet 1	5.80±0.21	−16		8.35±2.10	+11	
8	3.84	10.9	Inlet 1	2.90±0.05	0				
			Outlet 30	1.52±0.03		*	—	—	
			Outlet 1	0.46±0.02	−84				

numbers depend on three slides with 10 replicated counts of each.

The first three experiments were an attempt to determine the height above the flume bottom to which seston depletion occurred. The results (Table 3) show that there are significant differences in ATP–seston and bacterial numbers at heights of 1 and 30 cm above the bottom and that the depleted layer at the outlet end is at least 10 cm thick.

In five subsequent experiments, control samples were taken 1 cm above the mussels at the upstream station as well as at 30 cm above the mussels at the downstream station. These samples were expected to be identical, it being assumed that the water column was well mixed at the upstream end of the flume. The upstream control samples were found to be consistently higher in ATP and bacterial numbers. It is possible that systematic velocity differences between the two sampling points and therefore different departures from isokinetic sampling caused this discrepancy. Not enough is known of the hydrodynamic properties of the seston to analyze this source of error. Depletion was subsequently calculated, using the upstream sample as control.

The ATP results for all replicates of the experiments with blue mussels presented in Table 4 indicate depletion of 46–84%, which is significant at $p < 0.05$, with one exception (Table 4, exp. 7). Reductions in bacterial numbers within the benthic boundary layer were small and do not reach the 95% significant level.

Seston Depletion by Horse Mussels

As a consequence of beginning later in the year (October–November 1981), seawater temperatures were lower (Table 5) in the horse mussel experiments than in the blue mussel experiments.

Unlike blue mussels, it was not possible to determine if most

TABLE 5. Seston depletion over a horse mussel bed 3.6 m long and 0.3 m wide. Density was 164/m^2 and wet biomass 3.672 kg/m^2. ND = no difference; *$p < 0.05$.

Experiment	Mean current speed (cm/s)	Mean temp. (°C)	Sample position	ATP–seston $\bar{X} \pm$ SE (ng/L)	ATP–seston % depletion based on inlet 1		Bacterial nos. $\bar{X} \times 10^6$ ($\pm$SE)	Bacterial nos. % depletion based on inlet 1	
1	3.92	7.5	Inlet 1	8.94±0.50	0		7.42±1.88	0	
			Outlet 30	6.36±0.30		*	6.37±1.17		*
			Outlet 1	5.76±1.04	−36		4.49±	−39	
2	3.78	7.7	Inlet 1	16.38±4.88	0		6.00±1.57	0	
			Outlet 30	2.76±0.50		*	5.97±1.80		*
			Outlet 1	3.24±0.40	−80		3.66±1.18	−39	
3	3.35	7.8	Inlet 1	1.98±0.14	0		7.06±1.55	0	
			Outlet 30	1.53±0.21		ND	2.86±0.74		*
			Outlet 1	2.04±0.21	+3		3.35±0.36	−53	

horse mussels were actively pumping seawater during the experimental period. Visual examination showed that only approximately 10% of the individuals were gaping or showing other evidence of pumping activity. However, two of the three replicates completed showed significant ATP depletion (36–80%) of the input seston over the 3.6-m path length of the mussel bed, and in all three experiments, bacteria were reduced in numbers. In the case of experiment 2 (Table 5), ambiguity is introduced by the large difference between the two ATP–seston control samples, and the high depletion value observed should be treated with caution.

Discussion

Our results with blue mussels provide direct evidence in accordance with theory (Wildish 1977; Wildish and Kristmanson 1979) that ATP–seston, probably represented mainly by phytoplankters, can be depleted by passage over a blue mussel bed of sufficient length and mussel density. In only one of seven available experiments were bacteria removed from seawater by blue mussels. This result agrees with the findings of Wright et al. (1982), who explained that blue mussel gill structure is able to trap larger phytoplankton but not smaller bacterial cells. Other authors such as Birbeck and McHenery (1982) have shown that blue mussels can assimilate bacteria, rendering this issue controversial. Possibly, particle-attached bacteria are involved or blue mussels are able to adjust their gill microstructure when free bacteria are a potential food resource. Despite obvious pumping activity, blue mussels failed to remove seston in one replication (Table 4, exp. 7). Two of the three replications of the horse mussel experiment showed significant removal of ATP–seston and all three significant removal of bacteria, suggesting the trophic importance of bacteria to this species.

Our conclusions, based on a well-mixed boundary layer analysis, are supported by the results of experiment 3 (Table 3). ATP concentrations at 1, 5, and 10 cm above the bottom are statistically indistinguishable and they are also significantly lower than at the 30-cm level. The shape of the concentration distribution between 10 and 30 cm above the bottom (and of particular interest, the transition across the upper edge of the boundary layer) was not determined. The assumption that the well-mixed layer is roughly as thick as the flume boundary layer cannot be evaluated without additional measurements. Preliminary measurements of the plume produced by dye release from the flume inlet, with the flume bottom covered by cleaned, dead mussel shells, gave a plume thickness similar to that of the flume velocity boundary layer.

The ATP–seston measurements can be compared by the analysis presented in the appendix. For blue mussels, parameter P was estimated for each run using a clearance rate (ϕ) of 2 L/h, $\alpha = 0.8$, $a = 1.05$, and $b = 0.5$. The two latter terms are consistent with the velocity profile measurements, and although the term α cannot be determined accurately from those data, the value chosen is considered to be reasonable. The seston clearance rate for the mussel population studied is impossible to determine with precision, but the measurements of Møhlenberg and Riisgard (1979) suggest that the value used is typical of *Mytilus edulis*. The measured values of C as a function of P are shown as a dimensionless plot in Fig. 3.

Taken in the context of the approximations needed for the analysis, the results are encouraging, but with ATP depletions in experiments 6 and 8 off the general trend. If the control for experiment 6 is taken as the "outlet 30" concentration instead of the "inlet 1," C becomes 0.56 and therefore considerably closer to the other points. Since the "inlet" sample is much richer in ATP than any of the other control samples, there is reason to suspect it. The experiment 8 result is very different from that of experiment 7, which was done under similar conditions except that the inlet ATP values were very low.

One difficulty in interpreting these experiments is that the food resource utilized by suspension feeders is not known. Potential food resources transported as seston by tidal currents to suspension feeders include zooplankton, phytoplankton, bacterioplankton, detritus or sediment particles with their attached microflora, and dissolved organic substances. Hollibaugh et al. (1980) have demonstrated that blue mussels can ingest bacteria following radioactive labeling of the bacteria. Birbeck and McHenery (1982), using this technique, have shown that some species of bacteria were assimilated whereas others were unsuitable as food because the blue mussel lysosome was unable to rupture the bacterial cell wall. All species of bacteria were cleared from inhalant seawater and trophically unsuitable ones rejected as pseudofaeces, implying

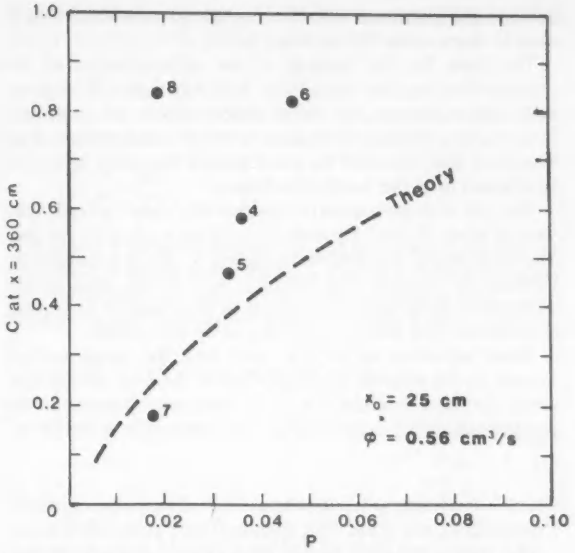

FIG. 3. ATP–seston depletion (C) observed at the outlet (X = 360 cm) as a function of parameter P.

selection by the mussel. The quantitative importance of bacteria in the diet of suspension feeders is not addressed in such experiments. Wright et al. (1982) attempted to do this by comparing the quantitative importance of phytoplankton and bacterial food sources in laboratory and field experiments with three species of active suspension-feeding bivalves, including the blue mussel. In their study, acridine orange epifluorescence direct counts were used to enumerate bacteria and measurement of chlorophyll *a* concentration to estimate phytoplankton abundance. The estimated pumping rate of blue mussels during the laboratory experiments of Wright et al. (1982) was 10 times lower than in the field, indicating a serious laboratory experimental artifact. During the field experiment, the level above the blue mussel bed at which inlet and outlet samples were taken was not indicated by Wright et al. (1982), rendering conclusions regarding clearance as suspect in view of our demonstration of the importance of the benthic boundary layer in seston depletion. The mean speed of seawater over the Massachusetts' estuary mussel bed investigated by Wright et al. (1982) can be calculated to be 18.45 cm/s with a path length of 46.5 m and mussel density of 3840/m^2, which should result in depletion; however, the roughness of bed and degree of wind and wave activity are not specified, making further calculation impossible.

A theoretical formulation of the seston balance (appendix) suggests that the seston concentration will decrease in the downstream direction if a unidirectional current passes over a dense mussel bed. This expectation is confirmed by the experimental results obtained here with both blue and horse mussels. Where seston concentration does decrease in this way as it passes over the mussel bed, we can expect mussel population growth (density times size adjusted average growth) to be greater at the upstream end of the bed. Decreasing seston concentration caused by mussel feeding may also provide an explanation for the discreteness of some mussel beds with the downstream part of the mussel bed being limited by the lack of seston as food. Both these hypotheses may be falsified in flume experiments of the kind described here if mussel growth is measured and mussel density controlled.

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Appendix. Experimental Design Considerations

The model of limited mixing proposed by Wildish and Kristmanson (1979) relies on hydrodynamic arguments described below.

Consider the idealized case of a steady flow across a flat sea bottom of considerable extent. The time-averaged dynamic properties are assumed to vary in the vertical direction but not laterally or in the flow direction. The flow near the boundary will be turbulent, and its exact nature will depend in part on the roughness characteristics of the sediment–water interface. In the simplest case, the density of the water will not change sufficiently over the thickness of the boundary layer to cause buoyancy effects. For the purposes of this discussion, the benthic boundary layer will be considered to be the region near the wall in which the mean velocity is significantly less than that of the overlying water (the "law of the wall region") and in which turbulent diffusion processes are dominated by shear turbulence generated by the sea bottom.

We will consider the case of a population of suspension feeders found in a bed that begins at a certain line normal to the flow direction and extends for a considerable distance downstream. The animals are found at a population density that is the same everywhere. As a first approximation, it will be assumed that the bottom roughness is unchanged by the presence of the animals and therefore that the flow field is unchanged in this region. Since seston is being removed from the water column, its concentration is reduced near the boundary and a depleted layer will develop. At a sufficient distance downstream, turbulent transport processes and consumption by the animals will become balanced and the vertical concentration of seston will not change further in the downstream direction.

The thickness of this layer of reduced seston concentration (a "concentration boundary layer") will be approximately the same as the boundary layer defined by the mean velocity distribution. In natural conditions, this dimension may reach a thickness of a few metres. The downstream distance required for a steady state to develop is of the order of decimetres.

Our earlier arguments (Wildish and Kristmanson 1979) are based on this simplified situation and demonstrate the relationship between the governing factors that will lead to limitation of growth. We appreciate that other factors may assume importance before these limitations arise, but we wished to examine the hydrodynamic and biological circumstances necessary for the mechanism to be applicable.

Hydrodynamic conditions present in the sea cannot be accurately duplicated, nor can sufficiently large beds of suspension feeders be formed in laboratory-scale apparatus. A flume flow can be created, however, in which the rate of vertical mixing is limited, though the rates will not be the same as in the ocean. Since there was no possibility of accurately reproducing the benthic boundary layer, it was decided to generate a laboratory flume flow, to observe its mixing characteristics, and to describe analytically the seston balance observed.

Preliminary calculations showed that a turbulent flow could be expected at the velocities of interest and that a turbulent boundary layer would develop, its thickness increasing in the downstream direction. Using relationships developed for turbulent flow over a smooth boundary (Schlichting 1960), the boundary layer 5 m downstream was predicted to be approximately 5 cm thick at a bulk velocity of 10 cm/s. Since the bottom of the flume would be hydrodynamically rough because of the presence of the mussels, the actual boundary layer was expected to be thicker. These calculations suggested that a flow about 30 cm deep would be sufficient to demonstrate a depleted boundary layer.

Above the upper limit of the developing boundary layer, vertical mixing is weak, and seston there, for all practical purposes, is unavailable to mussels. The upper limit of the boundary layer is not sharp, however, and its definition is not straightforward. In this work, mean velocity profiles were measured and used to define its position.

The Seston Balance

The transport and consumption of seston in the flume is accounted for in two ways. In the first, a seston balance is calculated for an arbitrary length of the flume. The boundary layer thickness is obtained from velocity measurements and the conditions for depletion are established.

In the second analysis, a differential balance is calculated and more general conclusions drawn about the relationships between the important variables. Again, experimental data is used to characterize the boundary layer.

The basis for the analysis is the approximation of the concentration profiles by simple step functions. At a given downstream distance the seston concentrations are characterized only by a thickness term and an average concentration. This simplified description of the concentration boundary layer will be referred to as the "well-mixed layer."

The flow near the bottom is considered to be well mixed in the vertical plane above the bottom for a distance yet to be defined. The thickness of this well-mixed layer, δ, can be estimated by considering velocity distributions. All of the fluid in the well-mixed layer will be assumed to be available to benthic animals but fluid above this layer will be unavailable.

Mean velocities vary from small near the rough surface formed by the mussels to a maximum in the undisturbed flow above the flume boundary layer. Its thickness increases in the downstream direction according to a relationship of the form

$$(1) \quad \delta = ax^b$$

where δ = thickness (centimetres), x = downstream distance (centimetres), and a and b are constants for a given flume flow.

The volumetric flow within the turbulent flume boundary layer can be estimated to be

$$\alpha U \delta \Delta Z$$

where U is the free stream velocity, ΔZ is the width of the flow, and α is a number between 0 and 1 that defines the average velocity in the well-mixed layer as a fraction of the free stream velocity.

In a mussel bed consisting of N individuals per square centimetre, assume that an average mussel filters water at the rate of ϕ cm^3/s^1. The ratio R of the total volume of fluid filtered by all mussels upstream of point x to total flow in the well-mixed layer is an indirect measure of the depletion that might be expected, assuming that all seston is filtered by the animal. Let

$$R = \frac{\phi N x}{\alpha U \delta}.$$

It is interesting to note that because the exponent b of equation (1) is less than unity, the ratio R will increase in the downstream direction. In other words, because more water is filtered by the suspension feeders than enters the boundary layer from above, the seston concentration in the boundary layer will decrease in the downstream direction.

For an experiment with a flume 3.5 m long, a mean velocity of 4 cm/s, blue mussels at a density of 0.1 cm^2, α = 0.8, a mussel clearance rate, ϕ, of 0.55 cm^3/s^1, and a concentration boundary layer thickness, δ, of 20 cm, R is found to be 0.30. These values are typical of our experimental conditions, and it follows that depletion of seston should be observed in the flume.

A differential balance can be written to derive a more exact description of the depletion process. Consider a laboratory flume and define a control volume of width Δz, length Δx, and thickness δ. The velocity profiles in the boundary layer are assumed to be similar at each downstream station, so that α is the same at all values of x, but δ increases with x. Since the flume boundary layer thickens in the downstream direction, there must be a net inflow from the free stream above. The rate at which the well-mixed layer thickens depends on the inflow from the main stream. The seston contained in this inflow is the only supply to the well-mixed layer. This volumetric flow into the

well-mixed layer is defined as $V\Delta x\Delta z$. Continuity requires that for an incompressible flow of constant density:

$$\alpha U\delta + V\Delta x = \alpha U\left(\delta + \frac{d\delta}{dx}\Delta x\right)$$

or

$$V = \alpha U \frac{d\delta}{dx}.$$

A seston balance for the well-mixed layer requires a term for the uptake of seston by the mussels. Putting c equal to the mass per unit volume of seston in the well-mixed layer and C_0 the same in the undisturbed flow above, the balance at any point x is

$$\alpha U\delta c + (V\Delta x)C_0 = \alpha U\left(\delta + \frac{d\delta}{dx}\Delta x\right) \times \left(c + \frac{dc}{dx}\Delta x\right) + \phi Nc\Delta x$$

or

$$\frac{d\delta}{dx}\left(\frac{C_0 - c}{C_0}\right) = \frac{\delta}{C_0}\left(\frac{dc}{dx}\right) + \frac{\phi N}{\alpha U}\left(\frac{c}{C_0}\right).$$

Replacing $(C_0 - c)/C_0$ by C, $\phi N/\alpha U$ by P, and δ by ax^b:

$$(2)\quad \frac{dC}{dx} = \frac{P}{a}x^{-b}(1 - C) - bx^{-1}C.$$

This equation can be integrated with the initial value $C = 0$ at $x = x_0$, corresponding to the flume boundary layer beginning at $x = 0$ and mussel bed at $x = x_0$. There does not appear to be a straightforward analytical solution and it was therefore solved numerically, using a Runge–Kutta–Fehlberg algorithm (Forsythe et al. 1977). Values of P ranging from 0.01 to 0.10 were used with values of a and b consistent with velocity profile measurements made in the experimental flume.

There are a number of assumptions used in this analysis, notably that of the well-mixed layer, and its equivalence to the boundary layer defined by the mean velocity. The latter assumption is not entirely consistent with the use of $x_0 = 25$ cm but the solution at $x = 360$ cm is not sensitive to it.

Short-Term Colonization Patterns of Lotic Macroinvertebrates

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To establish colonization of gravel-filled trays by aquatic invertebrates, we used concurrent simultaneous placement/sequential removal and sequential placement/simultaneous removal sampling schedules over 12-d intervals in a river of central Alberta. Colonization trays collected more taxa and greater numbers of animals than did unit area (Hess) samples of the natural substrate. There was no correlation between rank of abundance of the 10 most prevalent taxa found in colonization trays and in Hess samples. The two sampling schedules produced significantly different estimates of colonization rate and numbers for 6 of 10 abundant taxa. From hypothetical colonization curves derived for *Baetis tricaudatus* Dodds larvae from information on drift tendencies and settling abilities, we suggest that temporal pattern of tray colonization is strongly dependent on fluctuations in mean current velocity and short-term changes in upstream benthic density.

Pour estimer la colonisation de plateaux de graviers par les invertébrés aquatiques, nous avons utilisé des calendriers d'échantillonnage, se répétant tous les 12 jours, prévoyant d'une part le placement simultané suivi de retrait séquentiel ou, d'autre part, le placement séquentiel suivi du retrait simultané. Les essais ont été effectués dans une rivière du centre de l'Alberta. Le nombre d'espèces dans les plateaux était supérieur au nombre trouvé dans les échantillons (de Hess) des substrats naturels. Il n'y avait pas de corrélation entre le rang d'abondance, pour les 10 espèces les plus nombreuses, dans les plateaux de colonisation et les échantillons de Hess. Les deux modes d'échantillonnage conduisaient à des estimations différant de façon significative des taux de colonisation et des nombres d'individus pour 6 des 10 espèces les plus abondantes. Des courbes hypothétiques de colonisation obtenues pour les larves de *Baetis tricaudatus* Dodds à partir de données sur la dérive et l'aptitude à se déposer laissent à penser que la colonisation des plateaux en fonction du temps dépend fortement des variations de vitesse du courant et des modifications à court terme de la densité benthique en amont.

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Colonization studies have become an increasingly popular means of examining the composition of lotic communities. Sample substrates can be standardized, thus reducing intersample variability and permitting controlled tests of factors that might influence the benthic community (Rabeni and Minshall 1977; Culp et al. 1983). Colonization methods are particularly valuable in large rivers where standard benthic sampling is difficult. However, artificial substrates only approximate natural river substrate and do not always collect a sample representative of the benthic community. Because individuals of different taxa colonize at different rates, it is often unclear how long artificial substrates must remain in place before they become representative. Rosenberg and Resh (1982) comprehensively reviewed the benefits and shortcomings of artificial substrate methodology.

Traditionally, colonization studies have followed a "simultaneous placement" (SP) design; all samplers are introduced at one time and are subsequently recovered according to some predetermined schedule (Ulfstrand et al. 1974; Rabeni and Minshall 1977). This technique is appropriate for monitoring seasonal changes in species composition or abundance. However, where the colonization process itself is of interest (e.g. a comparison of colonization among treatments) a more suitable design is "simultaneous removal" (SR) (Shaw and Minshall 1980). By this method, samplers are periodically set in place and all are removed on the last day of the study. This ensures that all samplers are exposed to similar fluctuations in environmental conditions, at least immediately prior to recovery, and that animals collected are from the same sampling population. A shortcoming of this method is that stream perturbations can result in loss of all samples. Sheldon (1977) suggested that this problem might be minimized by estimating colonization rate and/or extrapolating equilibrial numbers of individual taxa from data collected over relatively short intervals.

Luttrell and Meier (1982) compared SP and SR designs over 12 wk and found substantial differences in accumulation of periphyton chlorophyll *a*, macroinvertebrate abundance, and standing biomass. Our study evaluates short-term colonization trends of individual taxa and examines the applicability of Sheldon's (1977) methods. Our specific objectives were to determine whether (1) colonization trays exposed for short periods collect a representative sample of the local benthic community, (2) SP and SR sample schedules reflect the colonization process equally well, and (3) the colonization process is significantly affected by temporal variation in current velocity.

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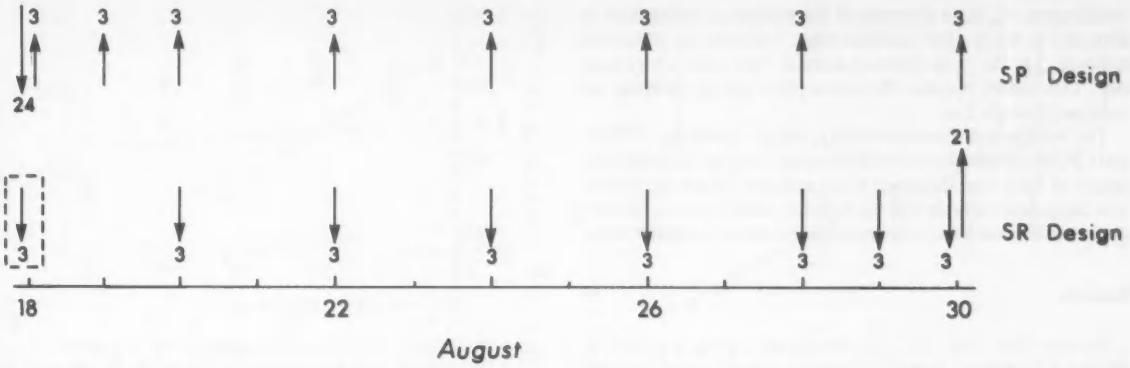

FIG. 1. Schematic outline of August sampling schedule indicating times of placement (downward pointing arrows) and removal (upward pointing arrows). Numerals indicate number of trays placed or removed. Enclosed arrow for SR design indicates trays not actually placed, but common to SP design.

Materials and Methods

The study site was a riffle on the Pembina River, central Alberta (55°36′N, 115°00′W). Here, the river is wide (65 m) and shallow (maximum depth 1.2 m), with substrate of smooth limestone cobble embedded in coarse sand. We have provided a detailed description of a similar site 800 m downstream (Ciborowski and Clifford 1983).

Colonization trays (25 × 25 × 10 cm) were made of 1.27-cm mesh galvanized wire screening filled by shovel with streamside substrate, a naturally occurring mixture of sand, gravel, cobbles, and detrital material. Experiments were conducted in May and August 1980. May trials were to last 30 d. On 14 May, 30 trays were placed in a 5 × 6 grid, each 1 m from any other. The area beneath each tray was thoroughly disturbed by kicking, and removing all large cobbles prior to placement. Depth and mean current velocity (v) (Ott C-1 current meter at 0.6× depth) above each tray were monitored. Five 0.1-m^2 Hess samples (Waters and Knapp 1961) were taken to estimate benthic density (B). Three randomly chosen trays were removed after 2 min and after 1, 2, 4, 6, 8, and 12 d. Flooding, which began on the 11th d, prevented further sampling and subsequently resulted in loss of all remaining trays.

Because preliminary data from May indicated very rapid colonization (densities of abundant taxa had stabilized after 2 d), August experiments were conducted over only 12 d. On 18 August 1980, 24 trays were placed at the same site, and 3 were harvested after 2 min. Benthic density in undisturbed areas was estimated from three Hess samples collected 1 and 11 d after initial placement. Groups of three trays were removed on each of days 1, 2, 4, 6, 8, and 10. Except on day 1, they were filled with new substrate and returned to their original locations. Other groups of three trays were set in place on days 11 and 12. All trays were removed on day 12. This procedure provided both SP and SR data series over the 12-d interval. Each consisted of three replicates of samples that had been in place 0, 1, 2, 4, 6, 8, 10, or 12 d (Fig. 1). The 12-d samples were common to both SP and SR data sets. One sample on each of days 8 and 10 of the SR series was partially lost during removal and was not considered in subsequent analyses.

Trays were recovered by sliding a three-sided aluminum box beneath them from upstream. A downstream dip net caught animals dislodged during harvesting. Each sample was transferred into enamelled pans at streamside. Stones were hand-rubbed to remove adhering material and discarded. The sample was then repeatedly flooded with river water, which, together with organic material, was poured through a 0.210-mm mesh net. Remaining inorganic material was carefully examined for stone-cased organisms before being discarded. Retained material was preserved in Kahles' fluid.

In the laboratory, samples were elutriated to remove any remaining inorganic material. Each sample was sieved into fine (FPOM; <1.00 mm) and coarse (CPOM; ≥1.00 mm) fractions. All animals were sorted beneath a dissecting microscope. The two fractions were then air-dried at room temperature and weighed to the nearest 0.01 g.

Data Analysis

To evaluate the similarity in taxonomic composition between colonization trays and Hess samples, we compared the 10 most abundant taxa collected by each method using Spearman's rank correlation coefficient. Rankings of abundance from colonization trays were estimated from total numbers collected on the 8th (last sampled preflood) and 12th d of May and August trials, respectively. These were compared with totals from the 5 May and 6 August Hess samples, respectively. Taxa considered accounted for between 83 and 97% of all individuals collected on the relevant sample dates.

Numbers of animals colonizing trays in August were compared by two-way analysis of variance (ANOVA, Dixon 1981). The two factors tested were time (days 1–10) and design (SP vs. SR). All data were transformed ($\ln(Y + 1)$) to equalize variances among groups. Ten taxa (see Table 1) were abundant enough to be analyzed by this method.

Sheldon (1977) suggested two empirical curves to which colonization data may be fitted: a power curve and an asymptotic ("equilibrium model") curve. We used the asymptotic model because the resulting regression parameters have biological relevance. We used least-squares nonlinear regression analysis (Dixon 1981) to describe colonization of individual taxa according to the formula

$$(1) \quad N_t = N_\infty(1 - e^{-m_e t})$$

where t = days of colonization, N_t = number of animals in colonization trays after t days, and N_∞ and m_e are regression

coefficients; N_∞ is an estimate of the number of animals to be expected in trays after inifinite time, whereas m_e represents turnover, i.e. the proportion of animals that leave a tray each day. This model requires the assumption that N_∞ and m_e are constant through time.

The influence of current velocity, depth, discharge, FPOM, and CPOM on numbers of individuals colonizing trays independently of time was examined using multiple linear regression. The dependent variable was the residual number of animals of a given taxon in each tray after nonlinear regression against time.

Results

Because the May trial was conducted during a period of declining discharge, current velocity initially decreased over the trays (Fig. 2). Flooding began on the 11th d and subsequently washed away all remaining trays. Water levels rose during August trials, exposing trays to substantially greater current velocities and water depths at the end than at the beginning of the experiment.

There was no pronounced systematic change in detritus concentration in colonization trays during the experiments, although some material accumulated during the first 2 d of SP trials (Fig. 3). Amount of FPOM in trays generally paralleled that in Hess samples. However, CPOM (decomposing twigs and leaves) was more abundant in trays than in undisturbed riverbed substrates.

Trays were rapidly colonized by most taxa collected in Hess samples. Between four and seven taxa were present in colonization trays at the time of placement (day 0, Fig. 4), but in most cases these were represented by only one or two individuals. Numbers of taxa in trays exceeded those in Hess samples after only 1 d. This difference persisted throughout the sampling periods and was evident for both designs.

Although Chironomidae dominated both Hess samples and colonization trays in all study trials, there was no significant association between order of abundance of taxa in trays and Hess samples ($n = 14$, $r_s = 0.16$, $p > 0.05$ in May; $n = 13$, $r_s = 0.33$, $p > 0.05$ in August). Thus, although Hess and tray samplers collected a similar variety of animals, their representation of abundant species differed markedly.

All but two of the taxa analyzed (*Isogenoides elongatus* (Hagen) and *Simulium tuberosum* Lundström) showed significant changes in number with respect to time ($p < 0.05$ by ANOVA). Because both *I. elongatus* and *S. tuberosum* were virtually absent from day 0 trays, we infer that saturation by these animals was achieved within 1 d. Significant differences due to design type were obtained for four taxa (*Baetis tricaudatus* Dodds, *Heptagenia elegantula* (Eaton), *I. elongatus*, *Alloperla* spp.). In all cases, greater numbers colonized during SR than during SP trials. Significant interaction effects were observed for *B. tricaudatus*, *Alloperla*, *S. tuberosum*, and Chironomidae. We interpret this also to be a function of differing colonization patterns among the two design treatments. In all, only 4 of the 10 taxa analyzed showed no design or interaction effect (*Ephemerella inermis* Eaton, *Rhithrogena hageni* Eaton, *Isoperla* spp., Hydropsychidae).

Colonization Patterns and Rates

Colonization by all abundant taxa was rapid. Densities of animals in trays after only 1 d approximated or exceeded those estimated from Hess samples. Colonization appeared to be more

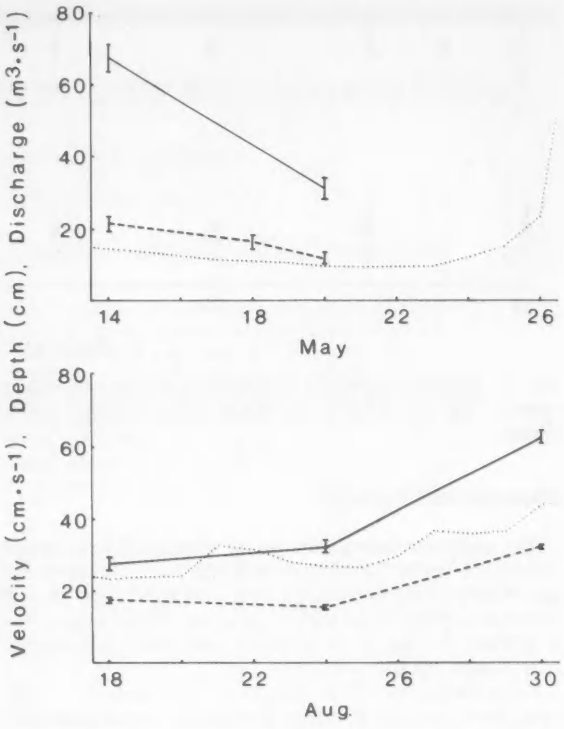

FIG. 2. Mean (±1 SE) current velocity (—) and depth (---) above colonization trays, and daily river discharge (···).

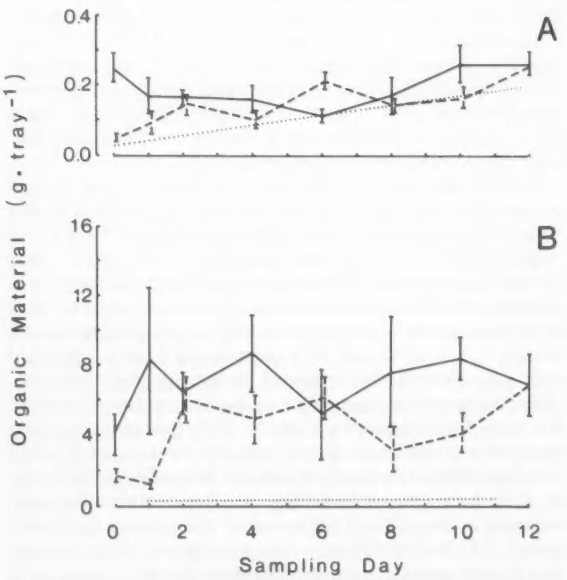

FIG. 3. Mean (±1 SE) amount of (A) FPOM and (B) CPOM in August colonization trays and Hess samples. —, SR design; ---, SP design; ···, Hess samples.

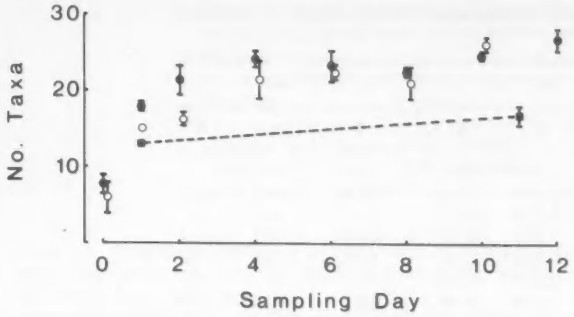

FIG. 4. Mean (±1 SE) number of taxa collected in August colonization trays (●, SR design; ○, SP design) and Hess samples (■).

rapid in May than in August; although, because life history stages differed, direct comparison is not strictly valid.

The equilibrium model described colonization patterns well in many cases, especially August SR and May trials. However, Hydropsychidae (Fig. 5A) and mayflies collected according to the SP design (Fig. 5B) tended to accumulate in a linear fashion with respect to time. Large stoneflies (*Claassenia sabulosa* (Banks), *I. elongatus* (Hagen), *Pteronarcys dorsata* (Say)) (Fig. 6) appeared in trays so rapidly that numbers did not vary over the course of sampling. *Baetis tricaudatus* (May), Hydropsychidae (May), and *Simulium* (Fig. 7) were unique in reaching maximum abundance within 1–4 d and then declining in numbers.

Regression estimates of m_e (emigration) ranged from zero for taxa whose colonization was a linear function of time to over 0.65 for mayflies that predominate in drift in the Pembina River (*R. hageni*, *E. inermis* in May; Table 1). Inadmissible emigration estimates ($m_e > 1.0$) occurred for taxa whose abundances peaked and subsequently declined. Estimates for SP observations were equal to or smaller than SR observations for all taxa analyzed except Chironomidae.

None of the other variables examined (velocity, depth, discharge, CPOM, FPOM) significantly reduced residual variation among samples for any of the taxa examined ($p > 0.05$). Evidently, any significant variability attributable to these factors was systematic and related to temporal rather than to spatial variation.

Modification of the Equilibrium Model

The SR sample schedule most frequently produced curves corresponding in shape to the equilibrium model, possibly because this design more closely met the model assumptions of constant numbers immigrating (K) and constant likelihood of emigration (m_e). However, because both K and m_e are largely determined by animals' drift tendencies (Townsend and Hildrew 1976; Williams and Hynes 1976) and drift is often strongly dependent on prevailing current velocity, which varied markedly during the trials, one should not expect these factors to be constant.

To estimate the sensitivity of perceived colonization pattern to temporal variation in current velocity, we modified the model to express its components in terms of drift as related to current velocity.

Sheldon's (1977) equilibrium model postulates that change in numbers in a colonization tray over time (dN/dt) reflects the

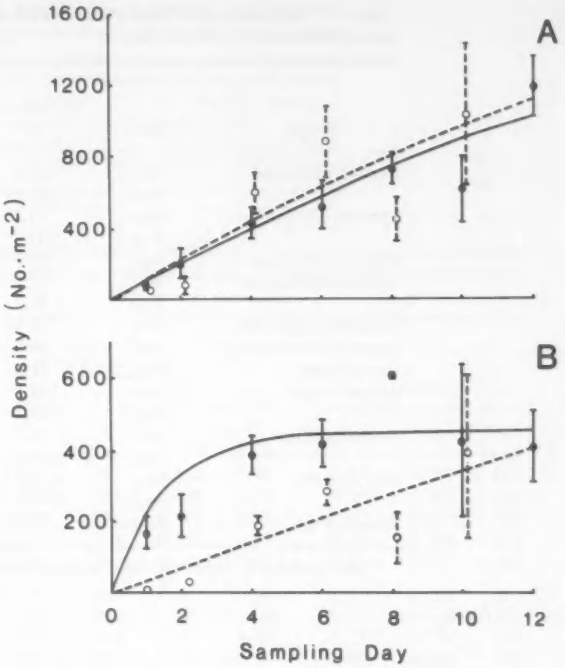

FIG. 5. Colonization of trays by (A) Hydropsychidae in August (B) *B. tricaudatus* in August. Lines are regression estimates; points and intervals are means (±1 SE). —, SR design; ---, SP design.

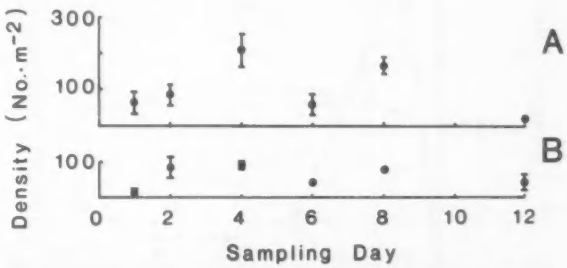

FIG. 6. Mean (±1 SE) density of (A) *C. sabulosa* and (B) *P. dorsata* collected in trays during May.

difference between number of animals immigrating per day (K) and the proportion of animals in the tray that emigrate (m_e):

$$(2) \quad dN/dt = K - m_e N.$$

(In the integrated form of the equation, $N_\infty = K/m_e$.) We will designate m_i and m_e as the daily proportion of animals that enter the drift in the river itself and from colonization trays, respectively.

The magnitude of K will depend on both the number of animals carried over a colonization tray and on individuals' ability to land successfully. We assume that the riverbed is uniform upstream of the trays and that the daily proportion of animals departing from the substrate (m_i) is homogeneous. The number of animals departing from each upstream region is thus $B \cdot m_i$ (upstream benthic density × proportion departing). Drifting animals settle randomly as they are carried downstream, with a species-specific settling rate (r). The equilibrial

TABLE 1. Regression coefficients and coefficients of determination relating number of individual macroinvertebrates per tray to time.

Taxon	Date	SP			SR		
		N	m_e	R^2	N	m_e	R^2
Baetis tricaudatus	May	12.0	1.936	0.02	—	—	—
	Aug.	248.8	0.009	0.46	28.6	0.683	0.28
Ephemerella inermis	May	70.1	0.673	0.35	—	—	—
	Aug.	500.0[a]	0.003	0.59	500.0[a]	0.003	0.54
Heptagenia elegantula	Aug.	133.0	0.006	0.48	10.6	0.187	0.36
Rhithrogena hageni	May	28.2	0.537	0.48	—	—	—
	Aug.	86.2	0.029	0.49	38.1	0.113	0.70
Claassenia sabulosa	May	8.8	0.679	0.22	—	—	—
Isogenoides elongatus	Aug.	4.5	0.229	0.09	5.1	0.666	0.21
Isoperla spp.	Aug.	31.4	0.260	0.39	28.1	0.375	0.42
Alloperla spp.	May	128.8	0.258	0.66	—	—	—
	Aug.	218.3	0.031	0.85	91.4	0.116	0.81
Hydropsychidae	May	22.3	1.176	0.13	—	—	—
	Aug.	183.4	0.041	0.55	172.1	0.040	0.75
Chironomidae	May	670.2	0.178	0.72	—	—	—
	Aug.	136.5	0.153	0.55	129.4	0.083	0.46
Simulium spp.	Aug.	23.6	0.906	0.03	24.1	2.562	0.01

[a]Value undefined; regression fitted to limit value of 500.

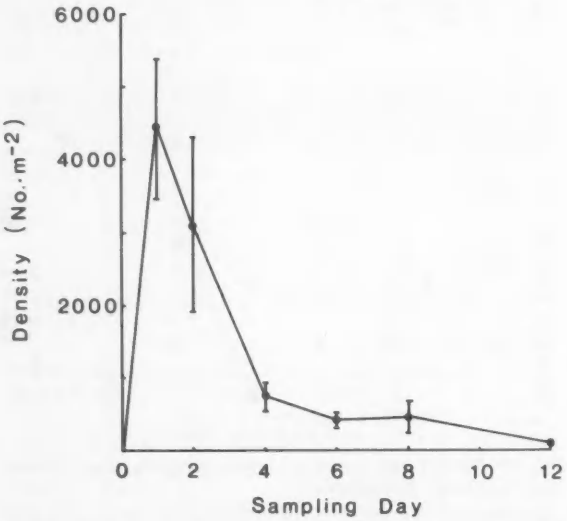

FIG. 7. Mean (±1 SE) density of *S. tuberosum* collected in trays during May. Lines were fitted by eye through means.

number of animals (Y_{max}) that will travel past a line across the river is (McLay 1970; Elliott 1971)

$$(3)\quad Y_{max} = B \cdot m_i \cdot r^{-1}.$$

Furthermore, the number of suspended animals that will settle per unit distance downstream is $Y_{max} \cdot (1 - e^{-r})$ (Elliott 1971). Hence,

$$(4)\quad K = Y_{max} \cdot (1 - e^{-r}) = B \cdot m_i \cdot r^{-1} \cdot (1 - e^{-r}) = B \cdot m_i \cdot \frac{(1 - e^{-r})}{r}.$$

Substituting in equation (2),

$$(5)\quad dN/dt = B \cdot m_i \cdot \frac{(1 - e^{-r})}{r} - m_e \cdot N.$$

Integration produces

$$(6)\quad N_t = B \cdot \frac{m_i}{m_e} \cdot \frac{(1 - e^{-r})}{r} \cdot (1 - e^{-m_e t}).$$

Current velocity can affect both r and m_e and hence N_t, as shown in the second and third groups of terms in equation (6). The relation between r and current velocity (v) is species specific but generally hyperbolic (Elliott 1971; Ciborowski and Corkum 1980; Ciborowski 1983a). Consequently, at low current velocities, r is relatively large and $(1 - e^{-r})/r$ is small. As current velocity increases, r rapidly decreases to an asymptote and $(1 - e^{-r})/r$ approaches 1. Thus, this term only becomes an important consideration at relatively low velocities ($<15\ cm \cdot s^{-1}$; J. J. H. Ciborowski, unpubl. data).

The relationship between v and immigration and emigration likelihoods is more complicated because drift probability can be either a positive or a negative function of current velocity, depending on the species and developmental stage examined. Many taxa are more prone to depart from the substrate at high current velocities than at low (Elliott 1967; Waters 1972); however, the reverse is often true for rheophilic organisms such as *Baetis* mayflies (Corkum et al. 1977; Ciborowski 1983b). If immigration and emigration are equal, the magnitudes of m_e and m_i will not affect the ultimate size of N after long periods, but they will affect the rate at which N approaches its maximum.

The formulation of equation (6) stipulates that N_t can never exceed B when $m_e = m_i$. In fact, the number of organisms in newly placed trays frequently exceeds benthic density in adjacent undisturbed regions (Rosenberg and Resh 1982; this study). Consequently, organisms must be, at least initially, less prone to depart from colonization trays than they are from other upstream undisturbed substrates (i.e. $m_e < m_i$). As colonization substrates become qualitatively more similar to undisturbed regions, m_e will become more similar to m_i. The rate of approach to equality will depend on the sensitivity of individual

TABLE 2. Equations relating daily proportion of *B. tricaudatus* individuals departing from substrate (m_i), instantaneous settling rate (r), and upstream benthic density (B) to mean current velocity (v).

Condition	Departure	Settling	Benthic density
Living	$m_i = 0.94 - 0.0087v$	$r = 8.02v^{-1.066}$	$B = -18.8 + 3.105v$
Dead	$m_i = -0.05 + 0.0063v$	$r = 3.43v^{-1.129}$	$B = -18.8 + 3.105v$

TABLE 3. Regression coefficients and coefficients of determination relating hypothetical numbers of living and dead animals colonizing trays to time under various current velocity regimes, and actual emigration rates (m_e), immigration rates (m_i), and upstream benthic density (B) on last day of sampling.

			Regression						Actual		
			SP			SR					
Condition	Benthic density	Velocity regime	N_∞	m_e	R^2	N_∞	m_e	R^2	m_e	m_i	B
Living	Constant	Constant	267	0.314	0.95	267	0.314	0.95	0.285	0.597	100
	Constant	Increasing	274	0.348	0.95	293	0.224	0.95	0.189	0.394	100
	Constant	Decreasing	266	0.230	0.94	258	0.387	0.91	0.333	0.697	100
	Increasing	Increasing	319	0.151	1.00	307	0.329	0.95	0.189	0.394	175
	Decreasing	Decreasing	242	0.516	0.71	210	0.312	0.95	0.333	0.697	68
Dead	Constant	Constant	253	0.141	0.99	253	0.141	0.99	0.141	0.296	100
	Constant	Increasing	324	0.076	1.00	205	0.247	1.00	0.211	0.442	100
	Constant	Decreasing	226	0.241	1.00	448	0.053	1.00	0.107	0.224	100
	Increasing	Increasing	600[a]	0.003	0.95	223	0.358	0.99	0.211	0.442	175
	Decreasing	Decreasing	238	0.459	0.95	600[a]	0.003	0.97	0.107	0.224	68

[a]Value undefined; regression fitted to limit value of 600.

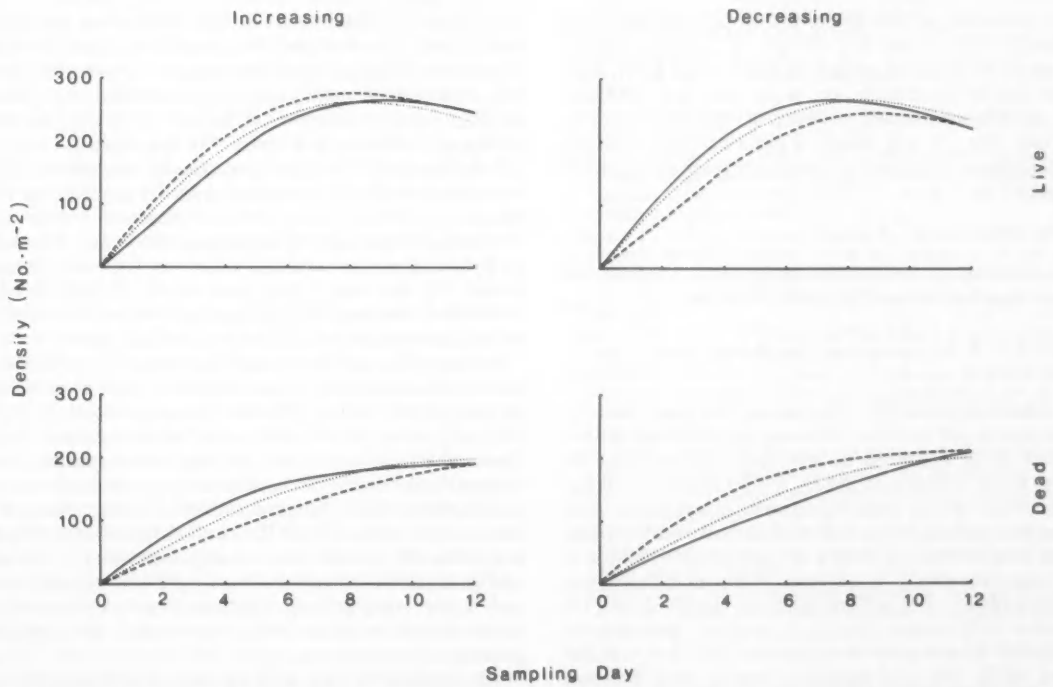

FIG. 8. Hypothetical colonization curves for living (upper) and dead (lower) *B. tricaudatus* larvae under increasing (left) and decreasing (right) current velocity regimes; benthic density (B) held constant. —, SR design; ---, SP design; ···, current velocity constant. Lines were fitted by eye through points.

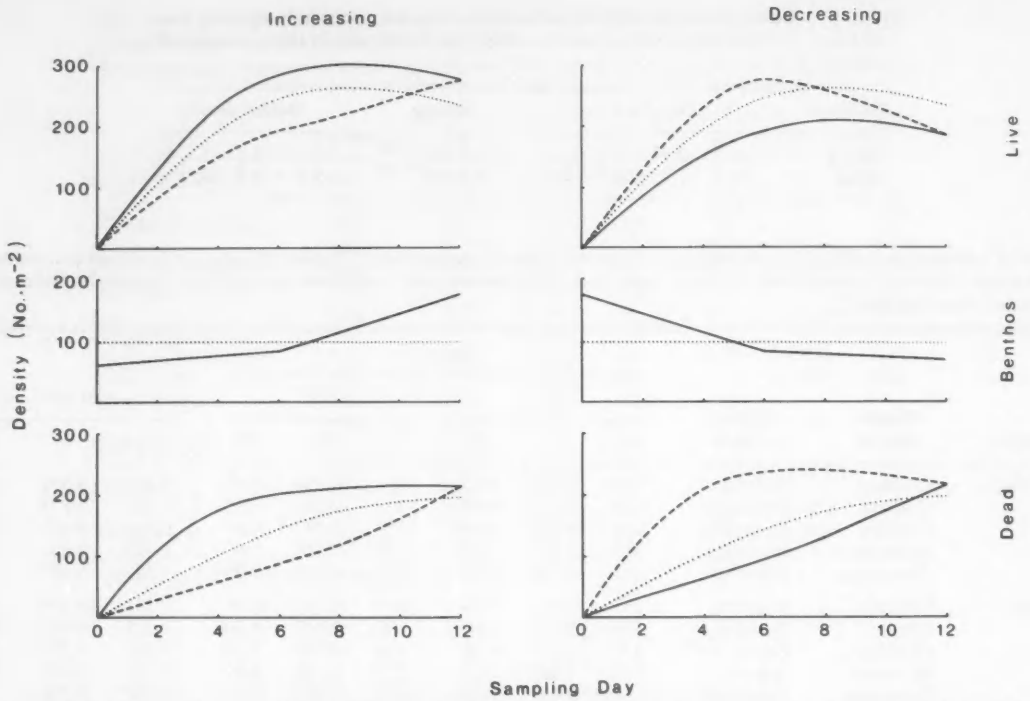

FIG. 9. Hypothetical colonization curves for living (upper) and dead (lower) *B. tricaudatus* larvae under increasing (left) and decreasing (right) current velocity regimes, with benthic density (B) varying as shown in centre panels. —, SR design; ---, SP design; ···, current velocity constant. Lines were fitted by eye through points.

taxa to environmental parameters and on potential inter- and intra-specific interactions. For example, *S. tuberosum* larvae predominate on clean rock surfaces in the Pembina River. Such surfaces rapidly accumulate silt, periphyton, and FPOM in spring, and this is presumably reflected in a rapid decline in the m_i:m_e ratio (Fig. 7). The pattern of decline of animals in this instance suggests an exponential relationship such that m_e might be expressed as

$$(7) \quad m_e = m_i \cdot (1 - e^{-ct})$$

where c is the instantaneous rate of change of tray suitability. In this case, equation (5) could be reformulated as

$$(8) \quad dN/dt = B \cdot m_i \cdot \frac{(1 - e^{-r})}{r} - m_i \cdot (1 - e^{-ct}) \cdot N.$$

To evaluate equation (8) under various flow conditions, we used laboratory and field data pertaining to the drift and settling ability of living and dead *B. tricaudatus* larvae from the Pembina River (Ciborowski 1983a, 1983b). Departure of live animals of this species from the substrate is a negative linear function of current velocity, and individuals can settle rapidly, whereas dead animals are carried off as a positive function of current and settle slowly, in a manner analogous to that of taxa with little mobility, such as Chrironomidae. Benthic density (B) also varies with current velocity, a result of larvae moving laterally in the river as water levels rise and fall (Ciborowski and Clifford 1983). We used equations relating daily departure likelihoods (m_i), instantaneous settling rates (r), and benthic density (B) of these animals to current velocity (Table 2) to predict hypothetical colonizing patterns during periods of increasing, decreasing, and constant mean curent velocity by both SP and SR procedures. Equation (8) was used to estimate expected colonization at 1-d intervals for 12 d, given the current velocity regime recorded during August trials (increasing flow). To determine the influence of decreasing velocity, the order of August readings was reversed. We also evaluated expected colonization pattern with current velocity constant, set at the overall mean value observed during August trials (38.25 $cm \cdot s^{-1}$). Benthic density (B) was 100 individuals·m^{-2} when held constant, or averaged 100 individuals·m^{-2} when allowed to vary. A substrate conditioning factor (c) of 0.05 was arbitrarily chosen. At this conditioning rate, m_e would be 90% of m_i within 46 d. The resulting colonization patterns were analyzed by nonlinear regression as described for field trials.

When current velocity was held constant, SR and SP designs provided identical results, and regression estimates of m_e were similar to actual values at the end of the experiments (Table 3). However, when velocity fluctuated, whether or not benthic density was permitted to vary, the shape of colonization curves changed appreciably. If benthic density was held constant, approximately linear increases in numbers were observed for dead animals analyzed by SP methods when current increased and by the SR method when velocity decreased (Fig. 8). Any change in current velocity produced regression estimates of m_e and N_∞ that were markedly different from values determined from constant velocity trials, even though all regressions accounted for a large amount of total variation (Table 3).

Permitting B to vary with current velocity magnified the differences between SP and SR designs (Fig. 9). In these cases, the state of animals (alive or dead) was of less consequence than the direction of change in current velocity. The SR design

produced large overestimates of m_e when velocity increased and underestimates when velocity decreased. The patterns generated for live animals under increasing current velocity conditions were consistent with those for *B. tricaudatus* larvae in August (Fig. 5B). Furthermore, the curve for live animals generated from the SP design during decreasing flow corresponded in shape to colonization patterns of most taxa during the May (SP) field trial.

Discussion

On a density per unit area basis, colonization traps were several times more effective in collecting aquatic invertebrates than Hess samples. This has been frequently reported (see Rosenberg and Resh 1982) and may partly reflect the greater amount of usable area available within the uncompacted substrate of the trays (Rabeni and Minshall 1977; Shaw and Minshall 1980). However, the taxonomic composition of the trays corresponded poorly with the local benthic community indicated by Hess samples. Colonization trays generally contained a wider variety of animals and substantially different proportions of individuals of common taxa. This probably represents the large contribution made by drifting animals from outside the immediate sampling area; these typically immigrate in proportions other than those represented by benthic samples (Waters 1972).

Simultaneous placement and simultaneous removal designs gave significantly different results for 6 of the 10 taxa analyzed in August. We anticipated that the problems of temporally varying physical conditions and changing community composition would be minimized by use of the SR design because all trays would have been exposed to the same current velocity regime and populations. The SR design did produce results more consistent with the equilibrium model (fewer turnover estimates were inadmissible). However, results of our hypothetical data manipulations suggest that this outcome was fortuitous. We postulate that the potential influence of daily variation in current velocity on both emigration likelihood and benthic density is so great that neither the SP nor the SR design alone can provide reliable measures of colonization patterns. Note, however, that the regression estimates of colonization variables for the two designs consistently enclosed the constant velocity values. Thus, concurrent use of the two sampling schedules should indicate conservative upper and lower limits of daily turnover. Furthermore, the magnitude of difference between the estimates of m_e should be a measure of the sensitivity of taxa to the variation in conditions during the study.

Our results lead us to question the validity of colonization samplers as tools to monitor lotic communities. Although trays collect many taxa, the individuals encountered include the fauna of habitats other than that in the immediate area. Furthermore, most taxa are overrepresented in trays and relative abundance corresponds poorly with densities in natural substrate. Although our trials were restricted to 12 d, there is little evidence that exposure of trays for longer periods improves the correspondence (Meier et al. 1979; Shaw and Minshall 1980; Rosenberg and Resh 1982). Because frequent sampling trips are expensive and there is an inherent danger of losing trays to floods or vandals, we question the utility of colonization sampling relative to standard quantitative (Hess) or even qualitative (kick net) sampling techniques. We concur with Rosenberg and Resh (1982) that colonization samplers are a necessary alternative when sampling habitats inaccessible by usual benthic collecting methods, but caution that precision of colonization sample estimates (variation among samples) should not be taken as indicative of accuracy (representativeness).

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Some Causes of Variation in Estimates of Mean Weights for *Hydropsyche* Larvae (Trichoptera: Hydropsychidae)

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Mackay, R. J. 1984. Some causes of variation in estimates of mean weights for *Hydropsyche* larvae (Trichoptera: Hydropsychidae). Can. J. Fish. Aquat. Sci. 41: 1634–1642.

I present monthly values of mean dry weight per larval instar for 1 yr for *Hydropsyche betteni* Ross, *H. bronta* Ross, *H. morosa* Hagen, *H. slossonae* Banks, and *H. sparna* Ross. Larvae of each species were collected from two or four sites in the Credit and Humber rivers of southern Ontario. Month-to-month changes in mean weight were more irregular in spring, summer, and autumn than in winter; summer values tended to have the highest standard errors. When growth was fast in summer, any one instar was constantly receiving low-weight individuals from the previous instar while losing heavier individuals to the next instar; successive monthly collections were sampling instars at various stages in the recruitment–loss continuum. Sexual differences were additional causes of wide ranges in weight in instar V; larvae destined to produce female pupae could be twice as heavy as those developing into male pupae. Because metamorphosis into males tended to precede that into females, heavier potentially female larvae were sometimes the only individuals collected during certain prepupal periods in summer. A mixture of cohorts and generations in summer also contributed to high variance. Winter weights, in contrast, were less variable from month to month and tended to have a smaller range per instar. When a particular instar was present in winter (December–March), its mean winter weight closely approximated the overall annual mean.

Je donne, pour une année, les valeurs mensuelles de la masse sèche moyenne des instars larvaires de *Hydropsyche betteni* Ross, *H. bronta* Ross, *H. morosa* Hagen, *H. slossonae* Banks et *H. sparna* Ross. Les larves de chaque espèce provenaient de deux ou quatre emplacements des rivières Credit et Humber dans le sud de l'Ontario. Les changements d'un mois à l'autre étaient plus irréguliers au printemps, en été et en automne qu'en hiver ; les valeurs d'été avaient tendance à avoir l'écart type le plus élevé. Lors des périodes de croissance rapide, en été, un instar recevait toujours des individus moins gros de l'instar précédant et perdait des individus plus gros au profit de l'instar suivant ; les prélèvements mensuels permettaient d'échantillonner les instars à divers stades dans le continuum des pertes de recrutement. Le sexe était une cause supplémentaire de variation à l'instar V ; les larves destinées à devenir des pupes femelles pouvaient peser deux fois plus que celles destinées à devenir des pupes mâles. Comme la métamorphose des mâles avait tendance à précéder celle des femelles, à certaines périodes prépupales de l'été on pouvait ne récolter que des individus plus lourds destinés à devenir femelle. Le mélange de cohortes et de générations contribuait également à la forte variance de l'été. En hiver, par contre, les variations étaient moindres et un même instar était plus homogène. Lorsqu'un instar particulier existait en hiver (décembre–mars) sa masse était voisine de la moyenne annuelle.

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Life history patterns of aquatic insect larvae are usually expressed in terms of numbers or proportions of individuals per age class or instar at regular intervals during the larval period (e.g. Mackay 1979; Benke and Wallace 1980; Cudney and Wallace 1980; Parker and Voshell 1982). In these terms, growth is expressed as the rate at which the population progresses from one instar to another. Alternatively, growth may be measured as the rate at which the population increases in biomass (e.g. Mackay 1972; Resh 1976).

Life history studies of hydropsychid caddisflies in southern Ontario (Williams and Hynes 1973; Mackay 1979, 1984) have considered only the proportions of larvae per instar. Therefore, in 1979 I began a year-long study of five species of *Hydropsyche* to determine the monthly mean weights of larval instars. By applying these mean values to the proportion of the population in any one of the five instars on the corresponding date, I would be able to calculate growth rate, in terms of biomass, in the population as a whole. Before I reached this second stage in the calculations, however, I became aware of some within-instar variability in weight that deserved consideration in itself. My data showed that month-to-month changes in mean dry weight per instar were more irregular in spring, summer, and autumn than in winter. This variability may have been unappreciated because few researchers have time to collect and weigh large samples of individual insects at frequent intervals (see also Ross and Wallace 1983).

Mean weight per instar or size class is used not only for growth rate studies but also to estimate annual production. The

size–frequency method of calculating production has been used in several recent hydropsychid studies (Benke and Wallace 1980; Cudney and Wallace 1980; Macfarlane and Waters 1982; Krueger and Waters 1983; Parker and Voshell 1983; Benke et al. 1984). None of these authors weighed individual larvae; mean weight per instar was calculated from a group of larvae in each instar and thus neither the range in weights nor the variance could be discussed. Ross and Wallace (1983) did weigh larval hydropsychids individually and noticed that biomass of later instars was "quite variable" but did not provide any error estimates. However, Ross (1982) tabulated annual means of these larvae, together with standard errors. The only observations of different weights within instars of *Hydropsyche* at different seasons were confined to the fifth (final) instar of certain species (Benke and Wallace 1980; Cudney and Wallace 1980; Ross and Wallace 1983), but even these seasonal values were not discussed in terms of range or variance.

Except for the seasonal grouping of fifth-instar hydropsychids, none of the production studies cited have described how larvae were selected from a year's collection for weighing. Were representative specimens weighed each month or was a small number of each instar picked at random from the total collection? When neither these details nor specific error estimates accompany mean weight data, the accuracy of the annual means cannot be evaluated. Current methods of estimating confidence intervals around size–frequency production values (Krueger and Martin 1980) do not account for errors in mean weight values, perhaps because these errors have not been appreciated.

My objective here is to present some of the causes of variance associated with mean weight estimates of *Hydropsyche* larvae so that such variability may be anticipated in studies of other taxa, and appropriate methods may be used to ensure that annual mean weights are calculated from the most reliable data. To this end I refer to five species at six sampling stations to demonstrate some trends common to a broad range of habitats and life cycle patterns.

Methods

The five species studied were *Hydropsyche betteni* Ross, *H. bronta* Ross, *H. morosa* Hagen, *H. slossonae* Banks, and *H. sparna* Ross.[1] Six sampling sites were selected in the Credit and Humber rivers of southern Ontario. Two sites (C1 and C2) were partially shaded third-order trout streams, two (C3 and H3) were unshaded fifth-order rivers draining agricultural areas, and two (HDam and PDam) were shaded third-order outflow streams from shallow impoundments. As five of these sites have been described before (Mackay 1979), only a few features will be detailed here.

Sites C1, C2, and C3 on the Credit river had maximum water temperatures in July and August of 22, 23, and 28°C, respectively. Sites PDam and H3 were on the main Humber River, while HDam was on Centreville Creek, a tributary of the Humber. PDam, not sampled previously, was at the Highway 50 (Ontario) crossing in Palgrave; it was chosen to resemble site HDam and thus to provide a pair of sites favouring *H. betteni*. Summer maxima on the Humber system were 23°C (PDam), 25°C (HDam), and 28°C (H3). Winter minima were 0°C at all six sites but PDam and HDam were generally free of ice whereas the other sites had extensive or complete ice cover between January and March 1980.

The study began with sampling at approximately 2-wk intervals in July and August 1979 from sites C1, C2, HDam, and PDam. Beginning in September 1979, all six sites were sampled on the same day at monthly intervals for 12 mo. Thick ice precluded sampling at C3 and H3 in February 1980. Several small boulders or handfuls of gravel and pebbles were lifted from each site using a D-frame pond net (mesh size 471 μm) to catch any escaping larvae. All visible larvae were handpicked live and preserved in Kahle's fluid (ethanol – formalin – glacial acetic acid – water = 15:6:1:30 parts by volume). Special effort was made to collect instars I and II, but these small larvae were difficult to detect in the field. Scrubbing of rocks could have damaged larval tissues (and reduced their weight) and microscopic sorting was impracticable given the number of samples to be processed on one day. All larvae were preserved within 6 h of collection.

Within 1 wk, and usually within 1–2 d, larvae were sorted, dried, and weighed. Several tests in the winter of 1978–79 had shown that mean dry weights of unpreserved larvae (killed in an ultrasonic cleaner within 30 s) did not differ significantly from larvae preserved in Kahle's fluid for 1 wk (Table 1). To ensure random selection of larvae for weighing, I spread out the sample in a petri dish. All undamaged larvae in a field of view through a dissecting microscope (×10 to ×20) were taken; I then moved the dish haphazardly to provide another view, and so on. Usually, all undamaged instars I, II, and III in the collection were weighed, but only a subset of larger larvae. Due to life history events, certain instars were naturally rare in some months.

Larvae that had been randomly selected for weighing were first suspended in water in an ultrasonic cleaner for approximately 30 s to remove silt trapped in cuticular setae. They were then dried at 55°C for 48 h, stored for at least 24 h in a desiccator, and weighed on a Cahn electrobalance (model 4100) to 0.01 mg (instars IV and V) or 0.001 mg (instars I, II, and III).

The final calculations were geometric means of dry weight because these are more appropriate than arithmetic means for weight changes due to growth (J. M. Elliott, Freshwater Biological Association, U.K., pers. comm.). No less than 3 and usually more than 10 individuals contributed to any calculation of mean weight. However, the 95% confidence limits around the means of 12 small samples reached such unrealistic extremes of weight for that instar that these data were omitted from the figures to make the results more conservative.

Results

The five species of *Hydropsyche* differed in abundance and life cycle patterns at the six stations. However, in spite of these contrasting spatial and seasonal distributions, the mean weight curves shared several characteristics.

The most irregular month-to-month changes in weight occurred between July and November 1979 at sites where

[1]The last four species have been considered by some authors to be in the genus *Symphitopsyche* (Ross and Unzicker 1977; Schuster and Etnier 1978); however, this name has now been judged inappropriate (Nielsen 1981; Schuster 1984). The name *Hydropsyche* is retained here for species in the former *bifida* group, now renamed the *morosa* group (Schefter and Unzicker 1984).

TABLE 1. Mean dry weights with standard errors of larvae killed in an ultrasonic cleaner compared[a] with those preserved in Kahle's fluid for 1 wk.[b]

Species	Instar	Ultrasonic		Kahle's	
		Mean dry mg ± SE	*N*	Mean dry mg ± SE	*N*
H. betteni	V	4.81±0.22	39	5.10±0.23	37
H. slossonae	V	5.44±0.50	7	5.24±0.68	7
	V	6.64±0.36	21	6.95±0.55	19
	IV	1.09±0.05	17	1.09±0.04	17
	IV	1.21±0.06	19	1.22±0.05	23
	III	0.303±0.013	5	0.300±0.019	6
H. sparna	V	4.16±0.23	17	4.40±0.19	16
	V	5.61±0.26	5	4.38±0.51	5
	V	4.16±0.17	20	4.17±0.13	22
	IV	0.70±0.03	15	0.70±0.03	16
	IV	0.68±0.03	5	0.69±0.05	6
	IV	0.63±0.03	15	0.55±0.03	15
	III	0.150±0.009	9	0.165±0.008	11

[a] *t*-tests between preserved and unpreserved mean weights in any one comparison showed no significant differences ($p > 0.05$).

[b] Larvae picked from the benthos were placed alternately (per instar) in each treatment.

species were bivoltine. These sites were HDam and PDam for *H. slossonae* and *H. betteni* (Fig. 1 and 2), C2 for *H. sparna* (Fig. 3), and HDam for *H. bronta* (Fig. 4). Site C3 and H4 were not sampled in July and August 1979, but some abrupt changes in weight can be seen between September and November 1979 in *H. bronta* at C3 (Fig. 4) and in *H. morosa* at C3 and H3 (Fig. 5), sites where these two species were bivoltine or trivoltine (Mackay 1984). In July and August 1980, when the sampling interval was 1 mo, weight changes appeared less abrupt than those during the corresponding period in 1979 when sampling was semimonthly. However, sharp increases or decreases in 1980 summer weights occurred in some species at certain stations, viz. *H. betteni*: instar III at HDam (Fig. 2); *H. sparna*: instars II and III at C1 (Fig. 3); *H. bronta*: instar III at C3, instras III, IV, and V at H3 (Fig. 4); and *H. morosa*: instars IV and V at H3 (Fig. 5).

The lowest weights of instars III, IV, and V were usually recorded between July and November; weights gradually increased during autumn, remained more or less stable in winter, and increased again in April. A few unexpected decreases in mean weight were observed in May, e.g. *H. slossonae* instar V at HDam and PDam (Fig. 1), *H. betteni* instar IV at HDam (Fig. 2), and *H. sparna* instars IV and V at C1 (Fig. 3). Pupation by the overwintering generation occurred between April and July 1980. Male pupae tended to mature before female pupae and were always lighter than females, usually almost half their weight (Table 2). Mature larvae could not be sexed, but the tendency for male-producing larvae to metamorphose earlier meant that samples of late-maturing larvae were more likely to include the heavy potential females. As a result, pronounced increases were sometimes observed in the mean weights of instar V (e.g. *H. slossonae* at C2 and HDam, Fig. 1).

At some sites where a population was bivoltine or trivoltine, samples of instar V in June 1980 contained individuals from the overwintering generation as well as individuals that had developed rapidly from eggs laid in May. Although most overwintering larvae could be recognized by the presence of epizooic protozoan colonies, separation of generations was not always possible. As a result, June weights for instar V had a wide range (e.g. *H. betteni* at PDam, Fig. 2) or were intermediate between May and July values (e.g. *H. bronta* at H3, Fig. 4). This inability to distinguish generations also contributed to variability in mean weight values of all instars between July and November 1979.

In contrast with the irregular rise and fall in mean dry weight per instar during spring, summer, and autumn, winter weight curves were smoother. Most species overwintered in instars III, IV, and V (exception: *H. slossonae* at C1, Fig. 1) and maintained their weights in these instars between December and March.

Another general trend is illustrated by the range of 95% confidence limits in Fig. 1–5. Standard errors tended to be higher in spring, summer, and autumn months than in winter. As these statistics incorporate sample size as well as variance, the trend was most obvious at sites where a species was abundant and sample size was relatively large. *Hydropsyche slossonae* at HDam (Fig. 1), *H. betteni* at HDam and PDam (Fig. 2), *H. sparna* at C1 and C2 (Fig. 3), and *H. bronta* at HDam (Fig. 4) all followed the trend. As stations C3 and H3 were not sampled in July and August 1979, nor in February 1980, the mean weight data there are too few to provide clear contrasts; however, all three species at these stations tended to have greater ranges in weight in spring and summer than in winter.

A final observation concerns the overall annual mean dry weight of an instar. When all the monthly means were averaged, the resulting overall means for instars III, IV, and V closely approximated the values recorded for these instars between December and March (see Fig. 1–5).

Discussion

Expressions of weight in terms of age classes are unavoidably subject to variability contributed by the range of weight within any one age class. When the number of age classes is small, as in

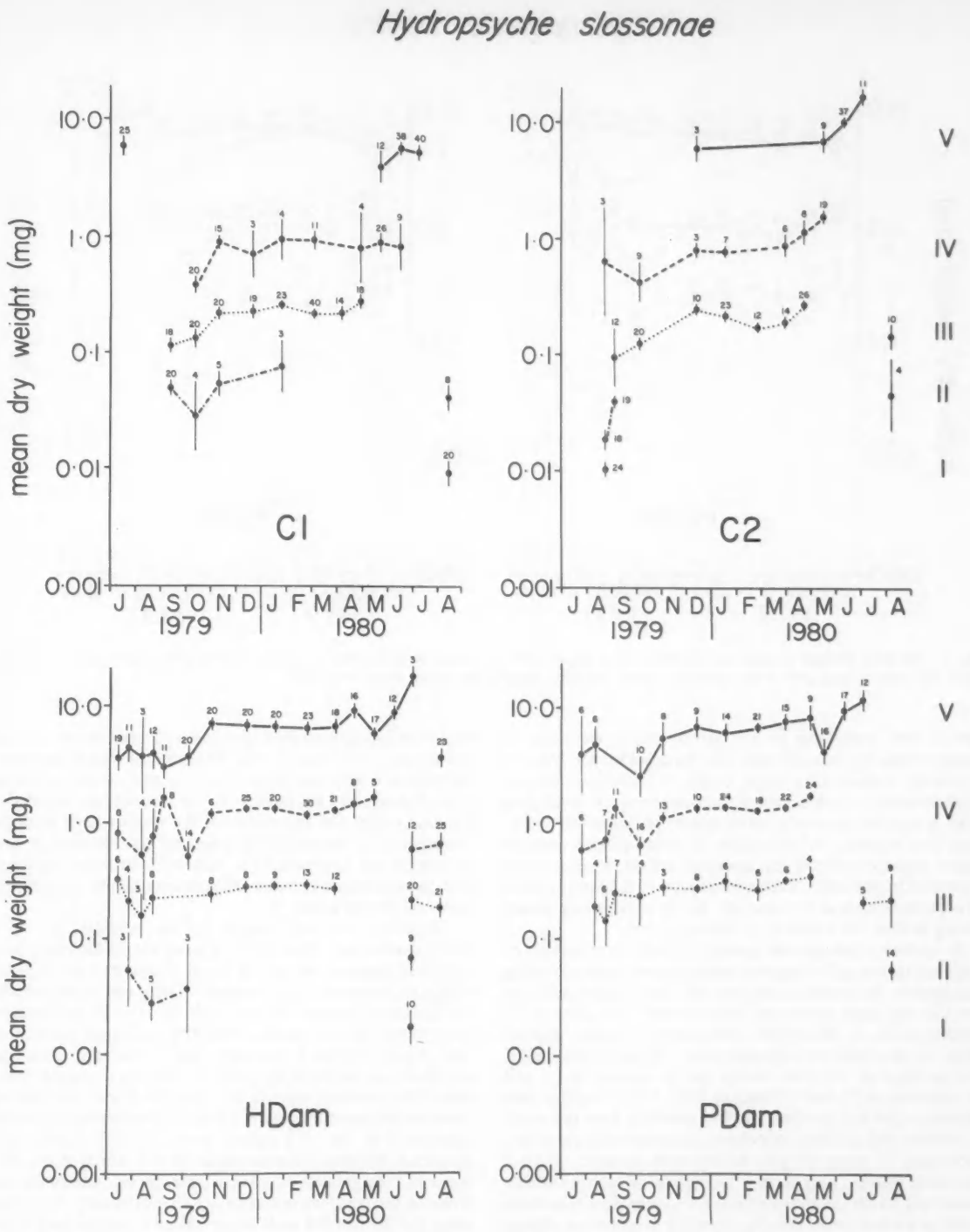

FIG. 1. Monthly changes in mean dry weights of five instars of *H. slossonae* larvae at four stations on the Credit and Humber rivers, in Ontario, 1979–80. Vertical lines show 95% confidence limits. Numbers weighed are shown above each point.

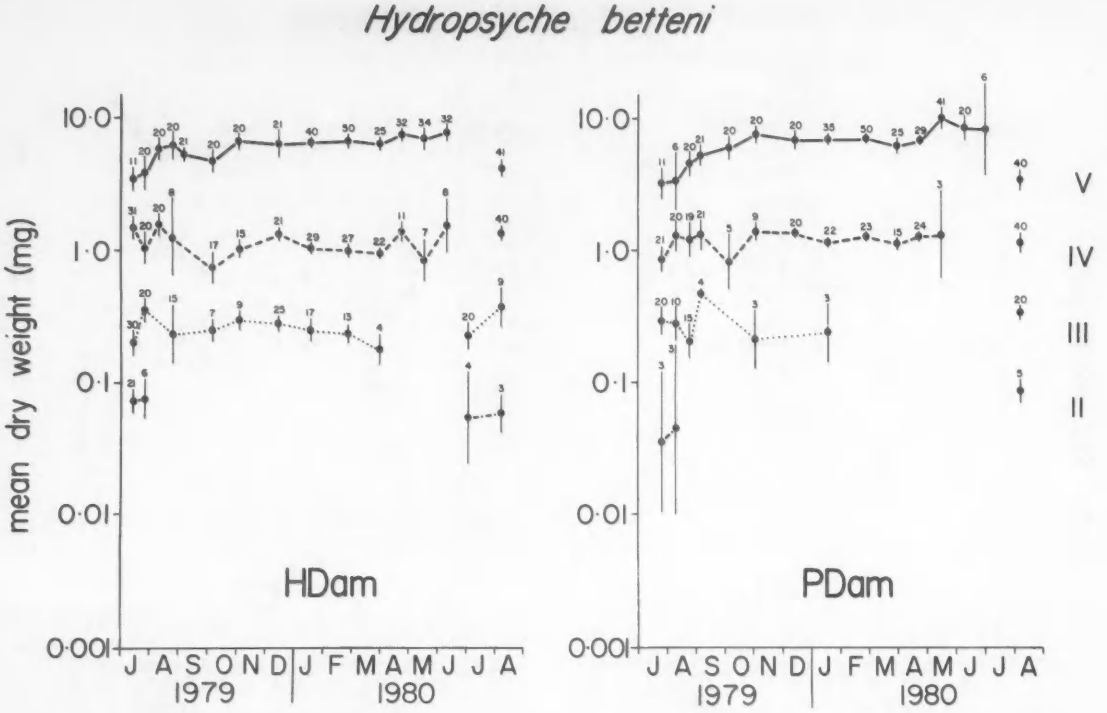

FIG. 2. Monthly changes in mean dry weights of four instars of *H. betteni* larvae at two stations on the Credit and Humber rivers, in Ontario, 1979–80. Vertical lines show 95% confidence limits. Numbers weighed are shown above each point.

insects with only three to five larval instars, the range in weight within any one age class may be considerable. When a population consists of a single cohort of individuals that all hatched within a week or two of each other and are developing more or less synchronously, mean weights per instar will tend to have low variance. A succession of rather precise monthly means probably reflects the changing weight of an average individual in that instar. Such a succession of weights is typical of caddisflies such as *Pycnopsyche* that grow relatively slowly during autumn and winter (e.g. Mackay 1972).

In contrast, *Hydropsyche* species typically have periods of adult emergence and oviposition lasting several weeks in spring and summer that result in long periods of recruitment although any one egg mass appears to hatch within a few days (R. J. Mackay and J. E. Rutherford, University of Toronto, unpubl. data). In the Credit and Humber rivers, *Hydropsyche* species are bivoltine or trivoltine except for *H. sparna* at C1 and *H. slossonae* at C1 and C2 (Mackay 1979, 1984). Rapid growth between April and October, frequent moulting from one instar to another, and a mixture of cohorts and generations result in a succession of mean weights having high variance. Even a univoltine species may have a succession of highly variable mean weights per instar that reflects population dynamics of the instar as a whole rather than the changing weight of an average individual in that instar. For example, the decrease in mean weight seen in *H. sparna* instar IV at C1 between August and October 1979 (Fig. 3) does not mean that the average individual larva was losing weight during this period. Instead, this decrease had two causes: the recruitment of low-weight individuals that had just moulted from instar III and the loss of older and heavier individuals to instar V. On the other hand, a marked increase in weight can be attributed to true growth within an instar that recently has neither received recruits nor moulted to the next instar. On any one date, the mean weight recorded corresponds to the particular point on the recruitment–loss continuum that happened to be sampled. The error associated with this continuum is the most important cause of variability in mean weight per instar.

Variability was also caused by the potential sex of a hydropsychid larva. This aspect of larval weight has rarely been addressed because the sex of larval hydropsychids (at least) cannot be determined by appearance. Pupae can be sexed easily but their weights have received little attention in the literature because they are not used in calculations of annual production. The pupal weights I obtained, and a distinctly biomodal distribution of prepupal weights (R. J. Mackay, unpubl. data), show that potentially female larvae in the final instar must be considerably heavier than the males. Maximum larval weights (presumed to be of females) were 25–30% higher than maximum weights of female pupae. In fact, larvae in the final instar had the greatest range in weight, approximately 20-fold from the smallest (sex unknown) to largest (female). This great range and the fact that male larvae tended to pupate earlier than females meant that collections made during the prepupal period were likely to display even more variance than usual in the weight of instar V.

The gradual rise seen in many mean weight curves during autumn occurred when moulting became less frequent. The

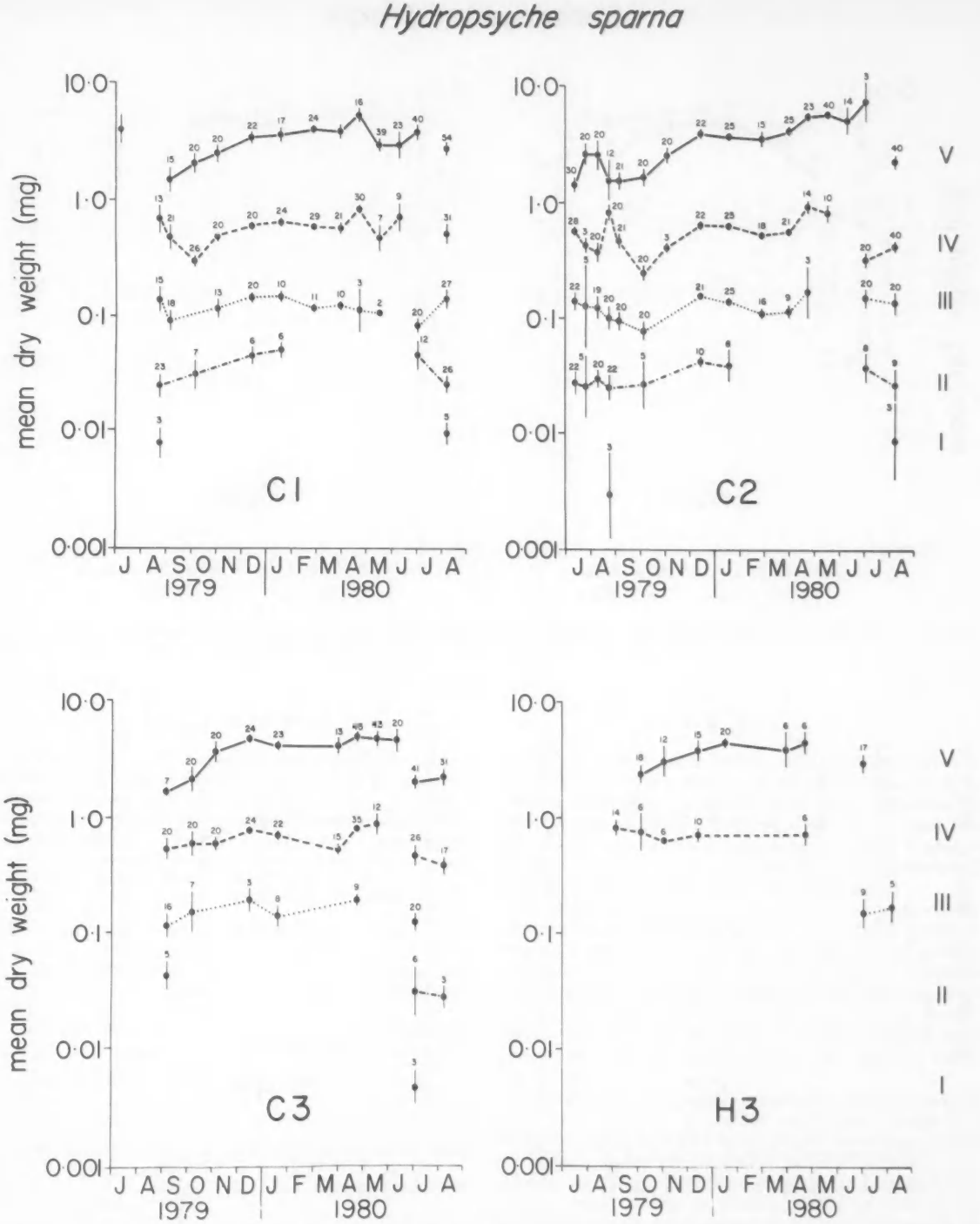

FIG. 3. Monthly changes in mean dry weights of five instars of *H. sparna* larvae at four stations on the Credit and Humber rivers, in Ontario, 1979–80. Vertical lines show 95% confidence limits. Numbers weighed are shown above each point.

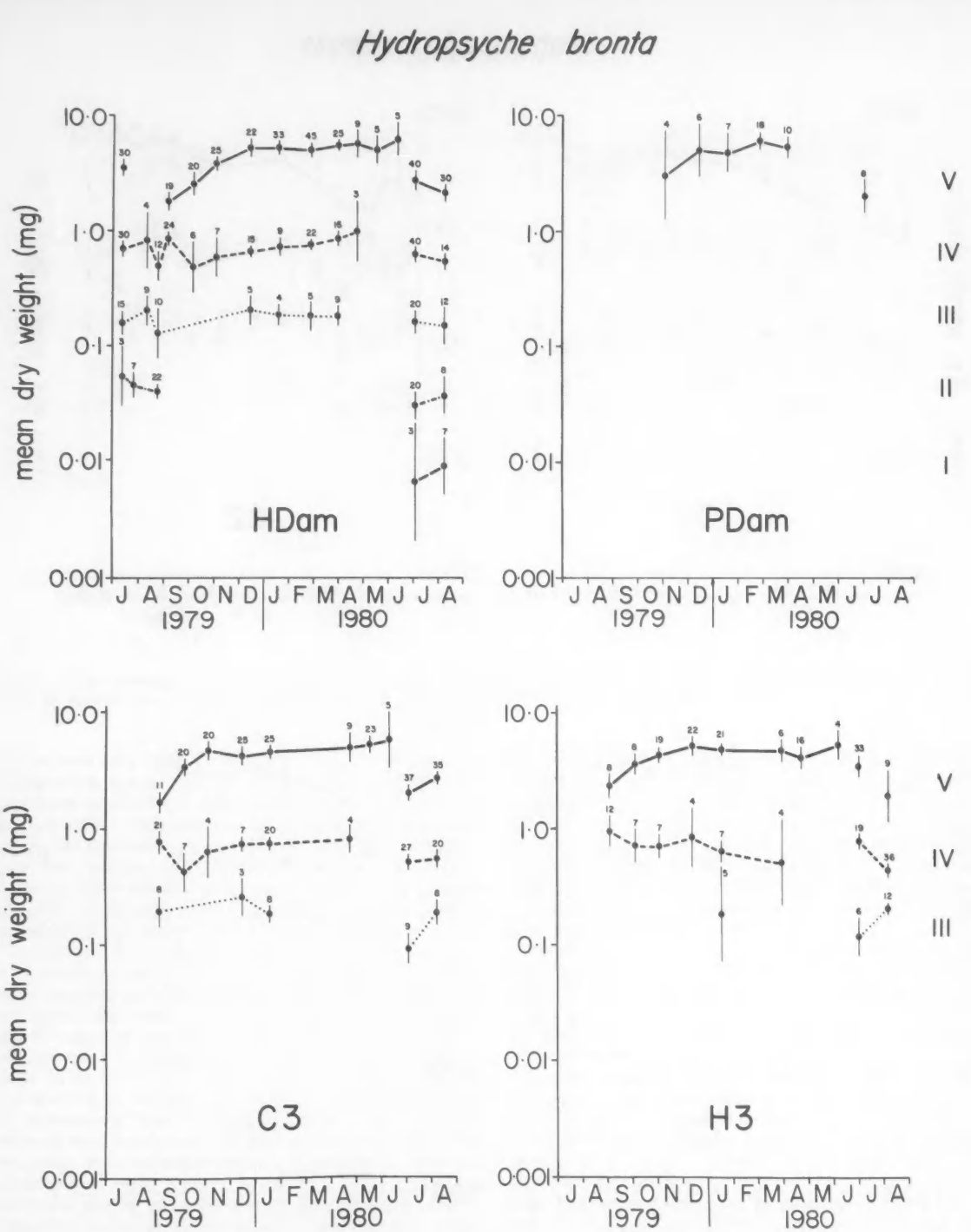

FIG. 4. Monthly changes in mean dry weights of five instars of *H. bronta* larvae at four stations on the Credit and Humber rivers, in Ontario, 1979–80. Vertical lines show 95% confidence limits. Numbers weighed are shown above each point.

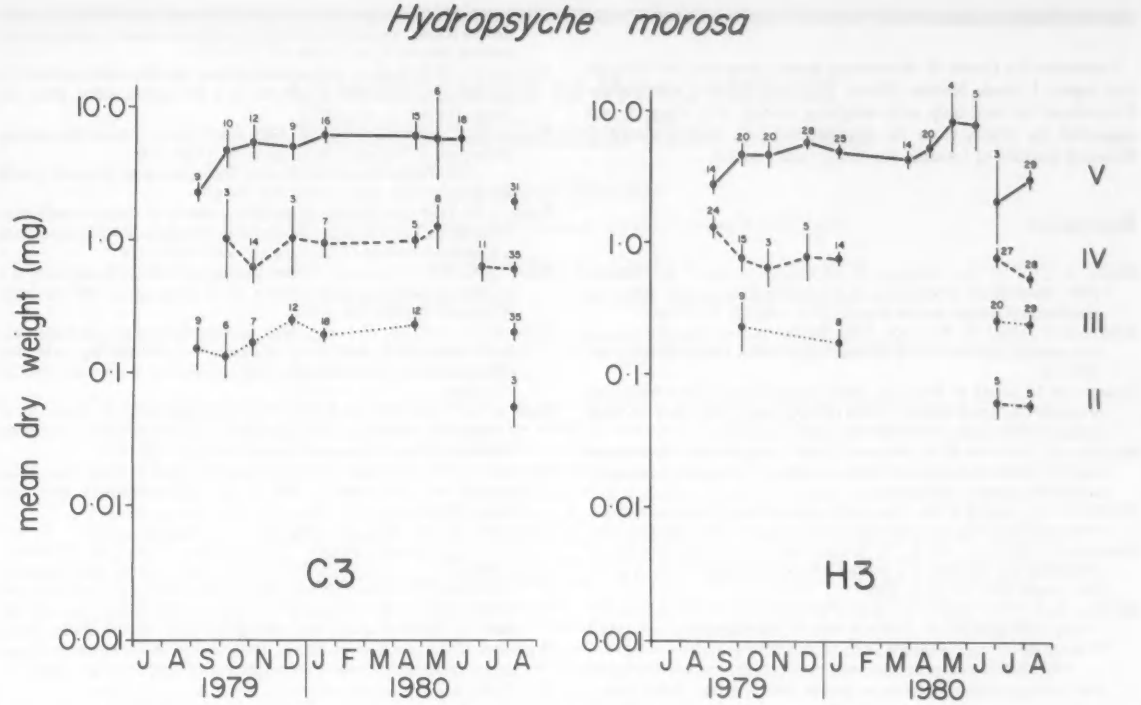

FIG. 5. Monthly changes in mean dry weights of four instars of *H. morosa* larvae at two stations on the Credit and Humber rivers, in Ontario, 1979–80. Vertical lines show 95% confidence limits. Numbers weighed are shown above each point.

TABLE 2. Individual dry weights or ranges in weight (number weighed in parentheses) of pupae from overwintering generations of *Hydropsyche* species in the Credit and Humber rivers.

Species	Month	Station	Female pupae	Male pupae
H. betteni	June	HDam	10.79–13.40 (3)	
		PDam	10.20–14.08 (13)	6.38–8.86 (5)
H. slossonae	May	HDam	9.53–11.43 (3)	5.57–7.37 (3)
		PDam	10.99	
	June	C2		4.63
		HDam	12.44	6.47–7.71 (2)
		PDam	12.29	
	July	C1		3.67–5.89 (2)
		C2	11.96–13.77 (5)	6.82–8.53 (3)
H. morosa	May	C3	11.34	4.81
		H3		4.54
	June	H3	7.53	4.20–5.43 (2)
H. bronta	May	C3	6.55–6.91 (4)	2.43–3.49 (3)
		HDam	6.20	4.13
		H3	7.61–8.87 (3)	4.64–4.84 (3)
	June	HDam	5.94	
		H3		4.76
H. sparna	May	C3	4.04–5.59 (3)	2.95–2.96 (2)
		H3	5.78	
	June	C3	5.88	

proportion of each instar between December and March is known to stay relatively constant in each species (Mackay 1979, 1984) and therefore, successive collections in the winter of 1979–80 were sampling a relatively stable age structure. The low variance associated with mean weight per instar suggested that individual larvae were gaining weight up to a certain level, which they maintained throughout the winter period.

The purpose of this paper has been to show how mean weights based on data obtained during periods of rapid larval growth or maturation may not provide the best estimates of average individual biomass at that time. My interpretations of variance obviously apply to species of *Hydropsyche* experiencing environmental factors similar to those in southern Ontario. But the same reasoning could be extended to other taxa that have periods of rapid growth alternating with relatively stable populations.

When monthly mean weights are required, or weight data are to be used for cohort production calculations, sample size should be greatly increased during periods when mean weight per instar is fluctuating on the recruitment–loss continuum. When an overall annual mean weight per instar is required, data from as many months as possible should be used in the calculations; exact details should be given on the sources of data. Alternatively, if the life cycle resembles those described for *Hydropsyche* species in southern Ontario, the mean weight per instar of overwintering stages may be a good estimate of the annual mean weight.

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Quantification of Wood Habitat in Subtropical Coastal Plain Streams

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To assess the importance of woody debris in two relatively unaltered Coastal Plain streams in the southeastern United States, a line intersect technique, developed by foresters, was used to estimate volume, mass, surface area, and spatial distribution. The ash-free dry mass of in-channel woody material was 6.5 kg/m^2 of stream channel bottom in the sixth-order Ogeechee River and 5.0 kg/m^2 in the fourth-order Black Creek. Most wood is located near the erosional bank in these meandering streams. These wood mass estimates are much higher than expected for middle-order streams and are similar to those from several small headwater streams in other regions. Due to their very low slopes (<0.02%), these streams appear to have insufficient stream power to move large woody material. Snag, or woody, habitat is the major stable substrate in these sandy-bottomed streams and is a site of high invertebrate diversity and productivity. In-channel snag surface area per square metre of channel bottom was 0.249–0.433 m^2 in the Ogeechee and 0.191–0.379 m^2 in Black Creek, depending on the hydrograph stage. With invertebrate biomass of 6.6 g dry mass/m^2 of snag surface, this results in an invertebrate biomass of at least 1.88 g/m^2 of channel bottom. Wood is also important to fishes, providing a rich source of invertebrate food, habitat, and cover. In addition to its obvious biological role, wood enhances the ability of a stream to process and conserve nutrient and energy inputs and has a major influence on the hydrodynamic behavior of the river. The quantification of wood habitat seems mandatory to assess past or potential impacts of snag removal on ecosystem processes in low-gradient streams.

Pour évaluer l'importance des débris ligneux dans deux cours d'eau de plaine côtière relativement inaltérés dans le Sud-Est des États-Unis, une technique d'échantillonnage linéaire, mise au point par des forestiers, a été employée pour estimer le volume, la masse, la superficie et la répartition spatiale. La masse sèche sans cendres de matière ligneuse dans le chenal était de 6,5 kg/m^2 de fond de chenal dans la rivière Ogeechee (6^e ordre) et de 5,0 kg/m^2 dans le ruisseau Black (4^e ordre). Presque tout le bois est situé près de la berge d'érosion dans ces cours d'eau sinueux. Ces estimations de la masse de bois sont beaucoup plus élevées que prévu pour des cours d'eau d'ordre moyen et elles sont analogues à celles qui proviennent de plusieurs petits cours d'eau (près de leur source), dans d'autres régions. En raison de leurs pentes très faibles (<0,02%), ces cours d'eau semblent posséder une force hydraulique insuffisante pour déplacer de grandes quantités de matière ligneuse. L'habitat du bois canard ou de la matière ligneuse correspond au principal substrat stabilisé dans ces cours d'eau à fond sableux et c'est le site d'une grande diversité et d'une grande productivité d'invertébrés. La superficie occupée par le bois canard, par mètre carré de fond de chenal, était de 0,249–0,433 m^2 dans la Ogeechee et de 0,191–0,379 m^2 dans le ruisseau Black, suivant le stade de l'hydrogramme. Avec une biomasse d'invertébrés de 6,6 g de masse sèche/m^2 de surface occupée par du bois canard, ceci produit une biomasse d'invertébrés d'au moins 1,88 g/m^2 de fond de chenal. Le bois est aussi important pour le poisson, puisqu'il fournit une source abondante d'aliments, un habitat et une couverture pour les invertébrés. Outre ses fonctions biologiques évidentes, le bois augmente la capacité qu'a un cours d'eau de traiter et de conserver les apports en matière nutritive et en énergie ; il a donc une influence considérable sur le comportement hydrodynamique de la rivière. La quantification de l'habitat boisé semble s'imposer pour évaluer les répercussions possibles de l'enlèvement du bois canard sur les processus de l'écosystème dans les cours d'eau à faibles gradients.

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Large woody debris, or snags, has been removed from rivers, especially low-gradient rivers, for centuries in efforts to create navigable waterways. Since such practices long preceded ecological investigations, the significance of wood in the structure and function of riverine ecosystems has not been widely appreciated. Many headwater streams are strongly influenced by the allochthonous inputs, primarily leaves, from their catchments (Cummins 1974; Hynes 1975). Woody debris also represents an input that may play an extremely important structural role in small streams (Marzolf 1978; Sedell et al. 1982). The purpose of this paper is to assess

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the importance of wood in larger (greater than third-order) streams, particularly as a habitat for invertebrates.

The importance of wood has been well documented in low-order streams with a relatively high gradient, including streams in New England (Bilby and Likens 1980), Oregon (Anderson and Sedell 1979; Naiman and Sedell 1979a; Triska and Cromack 1980), New Mexico (Molles 1982), southern Appalachians (Wallace et al. 1982), and some in New Zealand (Rounick and Winterbourne 1983). Woody debris has many diverse roles in headwater streams, such as rapid dissipation of the stream's energy (Bilby and Likens 1980); retention of other allochthonous organic matter (e.g. Bilby and Likens 1980), which influences both trophic and nutrient dynamics (e.g. Bilby 1981; Newbold et al. 1982; Melillo et al. 1983); providing valuable fish habitat (e.g. Marzolf 1978; Triska and Cromack 1980; Sedell et al. 1982); substrate for many stream invertebrates (e.g. Nilson and Larimore 1973; Anderson et al. 1978; Marzolf 1978); and food for some species of aquatic insects, which appear to be primarily xylophagous (Anderson et al. 1978; Anderson and Sedell 1979; Dudley and Anderson 1982; Pereira et al. 1982).

Vannote et al. (1980) predicted that the effect of riparian vegetation becomes insignificant in medium to larger (greater than fifth-order) streams, as either in-stream primary production or upstream inputs of fine particulate organic matter provide the major sources of energy. It logically follows that woody debris should play a minor role in larger streams as well. In larger streams of the Pacific Northwest (United States), woody debris is found in the riparian zone or deposited along river banks, but apparently plays a minor role in habitat formation in the main channel (Keller and Swanson 1979; Triska and Cromack 1980). The reduction in wood with increasing stream order appears to be true in streams greater than fifth order from a variety of biomes in which there are moderate to high stream gradients (0.09–0.6%) (Minshall et al. 1983).

In contrast with the findings of Minshall et al. (1983) and others, wood appears to be a major structural feature in middle-order streams (fourth to seventh) of the southeastern Coastal Plain. In these very low gradient streams (0.01–0.02% slope), "snag" substrates are important habitats for macroinvertebrates and sites of high secondary production (Cudney and Wallace 1980; Benke et al. 1984). Woody material affords a relatively stable habitat compared with the unstable, fine-grain sandy substrate that is characteristic of Coastal Plain streams. Furthermore, several species of game fish forage almost exclusively on invertebrates associated with these woody substrates (Benke et al. 1979; Benke et al., unpubl. data.). Submerged wood plays a similar role in many of the larger streams of the Amazon Basin (Fittkau et al. 1975; Fittkau 1982; Sioli 1982), and it may be an important habitat in most low-gradient streams unless snagging operations are a part of their recent history. Sedell et al. (1982) suggested that wood was a major structural feature of many large rivers before man removed this material for navigational purposes.

The major objective of this study was to provide estimates of woody debris and its spatial distribution in two middle-order (fourth and sixth) southeastern Coastal Plain streams. To accomplish this objective requires estimates of wood volume and mass, which are important from a structural standpoint, and wood surface area, which is the most appropriate measure of available invertebrate habitat.

Study Sites

The Ogeechee River has a drainage basin of 14 335 km^2 and flows southeasterly across the Coastal Plain to the Atlantic Ocean. The total length from origin to mouth, about 24 km south of Savannah, GA, is about 390 km. The study site is located about 63 river km from the mouth at an elevation of approximately 6.1 m a.s.l. where the sixth-order river drains an area of approximately 7000 km^2. Average annual precipitation over the basin is 117 cm, and average basin runoff is approximately 28 cm. There is little topographic relief at the study site, stream gradient is 19.6 cm/km, and the river meanders within a broad, heavily forested, lowland flood plain swamp, exceeding 1 km in width on either bank of the stream. Mean channel width is 33 m. Mean annual discharge at the study site is 67.7 m^3/s with long-term extremes approximately 850 m^3/s (1936) and 3.7 m^3/s (1954) (United States Geological Survey 1982).

The wide forested flood plain of the Ogeechee forms a buffer strip between the river and both farm lands and extensive pine forsts within the basin. The dominant trees associated with the banks and adjacent flood plain forest include baldcypress (*Taxodium distichum*), sweetgum (*Liquidambar styraciflua*), water oak (*Quercus nigra*), black gum (*Nyssa sylvatica*), water tupelo (*Nyssa aquatica*), willow (*Salix* spp.), and several pines (*Pinus* spp.). Willows dominate point bars whereas any of the other tree species may be found along the erosional banks of the meandering, convoluted path of the stream. The stream bed consists primarily of fine sandy substrate with some pebble and small gravel substrate in areas of higher velocity.

Black Creek is a fourth-order tributary of the Ogeechee, entirely located on the Coastal Plain, and drains an area of 767 km^2. Our study site is approximately 6.2 m a.s.l. with a drainage area of approximately 755 km^2 and a stream gradient of 29.8 cm/km. The flood plain varies from only a few metres in width, where forested bluffs rise 3–6 m in height above the stream, to 1.7 km in width. The bottom substrate consists primarily of white sand and occasional outcrops of sandstone. Mean stream width during low flow conditions is approximately 10 m; however, this width increases rapidly during rising water as sloping sand bars are inundated (bankfull width = approximately 21 m). Prior to 1980, Black Creek was gaged only periodically and discharge records range from zero flow (1954) to over 45 m^3/s (1980) (United States Geological Survey 1982). The Black Creek flood plain is also heavily forested, and with the exception of *Salix*, which is absent along Black Creek, dominant trees are similar to those of the Ogeechee.

Materials and Methods

In order to estimate wood volume and wood surface area, we utilized the line intersect technique, originally developed by Warren and Olsen (1964) to estimate logging residue. The theory of the method is explained by de Vries (1974). He pointed out that if a straight line of length L is run through a population of elements (such as logs), and if n elements intersect the vertical plane created by this line, then

$$(1) \quad \hat{X} = (\pi/2L) \sum^{n} X_i/l_i$$

is an unbiased estimator of the mean quantity of these elements per unit area, where X_i is the true value of any characteristic

(such as log volume) of the ith element and l_i is a uniquely defined length (such as log length) associated with that element. de Vries (1974) showed that an estimate of log volume per unit area can be derived from the above equation by substituting log volume, $(\pi/4)d_i^2 l_i$, for X_i, where d_i is the diameter of a stem intersected by the line. This results in the expression

$$(2) \quad \hat{X}_v = (\pi^2/8L) \sum^{n} d_i^2.$$

Thus, for a transect of length L, all stem diameters d_i are measured and can be used to calculate the volume of wood per unit area. Notice that lengths of stems (l_i) do not need to be measured, since they cancel out in the equation. Van Wagner (1969) provided a clear discussion of the actual procedure for taking measurements, and he pointed out that the method assumes that all stems are lying on a horizontal surface. However, he showed that the error is less than 10% if the angle is 25°.

In order to estimate wood surface area, a second equation was derived based on the principles elucidated by de Vries (1974). The expression for the surface area of a cylinder, $X_i = \pi d_i l_i$, rather than volume, was substituted into equation 1 to obtain

$$(3) \quad \hat{X}_{sa} = (\pi/2L) \sum^{n} (\pi d_i l_i)/l_i.$$

Again, l_i's cancel out and the expression simplifies to

$$(4) \quad \hat{X}_{sa} = (\pi^2/2L) \sum^{n} d_i.$$

Sampling was conducted during the lowest stream discharge possible, when wood can be measured accurately and safely. To determine the horizontal distribution of wood, a nylon line marked at 1-m intervals was stretched across the stream channel at a height 1 m above the water surface, and stem diameters (both living and dead) were tabulated within each metre mark along the transect. Depth was measured every 2 m along each transect in the Ogeechee and every 1 m in Black Creek. Stem diameters were tabulated at three heights: below the water surface, 0–1 m above the surface, and 1–2 m above the surface. The 2-m elevation was near the height of the bank and therefore, sampling above 2-m elevation was not feasible. However, stem abundance within the channel decreases considerably from the stream bottom through increasing heights and appeared negligible above 2 m.

Samples were taken in November 1982, August 1983, and September 1983 in the Ogeechee River and on 9–10 September 1983 in Black Creek. Twenty-one transects were measured across each stream, which encompassed a distance of 3 km in the Ogeechee and 1 km in Black Creek, in areas where we have sampled benthic and snag populations of invertebrates. Whereas representative sampling was conducted, completely random samples were impossible. First, underwater stems were measured by snorkeling, and the combination of reduced vision and current limited underwater sampling to <2 m depths, and second, snorkeling was too hazardous when attempting to measure submerged stems of some of the extremely large fallen trees.

To assess the distribution of wood across an "average" transect in the Ogeechee River, the percentage of wood volume was determined within each 10% increment of stream width for each of the 21 transects at each of the three heights. The mean percentage of wood within each 10% increment for each height was calculated, and the mean values were plotted for this "average" transect.

A major objective in estimating wood surface area per square metre of stream bottom is to convert invertebrate densities per square metre of wood surface to densities per square metre of stream bottom. Since the amount of wood inundated changes continuously with the rising and falling hydrograph, secondary production estimates, based on invertebrate densities on snags, require a reasonable estimate of wood habitat availability at different water levels. Mean values for wood surface area at the three transect heights were used to develop predictive equations of surface area for a given river height and to estimate temporal patterns of wood habitat availability. Cumulative wood surface area at water level (submerged) and 1 and 2 m above the water was plotted against stream height (at United States Gelogical Survey gaging station). Weekly mean stage heights for the Ogeechee River and Black Creek (United States Geological Survey, unpubl. data) were used in these regression equations to obtain weekly estimates of submerged snag surface area per square metre of channel bottom over a 92-wk period.

On 14 December 1981, 19 snag samples were collected from the Ogeechee River. Organisms were removed from each snag and the wood surface area of each snag was estimated (see Benke et al. 1984 for details of procedure). After identification and enumeration of aquatic insects, densities of all taxa were converted to numbers and biomass per square metre of snag surface. Mean standing stock values were derived for the major functional feeding groups (modified after Cummins and Klug 1979).

Specific gravity measurements were required to convert our wood volume estimates to mass per square metre of channel bottom. A series of wood samples ($n = 50$) ranging from small twigs (4 mm in diameter) to large logs (>500 cm in diameter) were sampled to obtain specific gravity estimates. Dimensions of wood <3 cm in diameter were measured with vernier calipers to the nearest 0.1 mm to obtain volume. Larger limbs and tree trunks were cored with an increment borer (5.5 mm in diameter) and length (40–150 mm) of each core was measured with calipers. Each sample was oven-dried (50°C) for 5 d, desiccated for 2 d over $CaSO_4$, and weighed. Samples were then ashed at 500°C for 8 h and reweighed to obtain ash-free dry mass (AFDM). This procedure is intended as a rough estimate of wood mass in the streams based on the product of volume estimates, derived by line intersect, and specific gravity (using AFDM).

Results

Wood Surface Area and Volume

In the Ogeechee River, most wood (surface area and volume) is submerged, with each succeeding height being significantly less ($P < 0.05$) than that below it (Table 1). Wood surface area at the 0–1 m height is 58% of that found below the surface. At the 1–2 m height, it declines to 16% of that below the surface. The decline in wood volume is even sharper: 40% (0–1 m) and 7% (1–2 m).

In Black Creek, there is little difference between amount of submerged wood and that at the 0–1 m height (Table 1). Wood declines sharply at the 1–2 m height to 7% of the submerged surface area and 5% of the submerged volume. At low water,

TABLE 1. Wood surface area, volume, and mean diameters (±95% CI) in the Ogeechee River and Black Creek for submerged wood and wood 0–1 and 1–2 m above the surface of each stream. Based on 21 line intersect transects for each stream.

Item	Ogeechee River				Black Creek			
	Submerged	0–1 m above surface	1–2 m above surface	Total	Submerged	0–1 m above surface	1–2 m above surface	Total
Surface area of wood (m^2)/m^2 of stream bottom	0.249 [a] (±0.078)	0.145 [b] (±0.045)	0.0398 (±0.0146)	0.433	0.380 [b] (±0.103)	0.162 [b] (±0.057)	0.026 (±0.011)	0.568
Volume of wood (m^3)/m^2 of stream bottom	0.0101 [b] (±0.0042)	0.0040 [b] (±0.0019)	0.0007 (±0.0004)	0.0148	0.0110 [c] (±0.0049)	0.0056 [b] (±0.0037)	0.00003 (±0.0002)	0.0168
Surface area of wood (m^2)/linear m of stream	7.17 [a] (±1.99)	4.63 [b] (±1.65)	1.35 (±0.52)	13.16	3.76 [c] (±0.98)	3.26 [b] (±1.06)	0.530 (±0.230)	7.55
Volume of wood (m^3)/linear m of stream	0.328 [b] (±0.126)	0.131 [b] (±0.061)	0.027 (±0.009)	0.485	0.113 [c] (±0.054)	0.108 [b] (±0.065)	0.005 (±0.004)	0.227
X diam (cm)	3.53 [b] (±0.78)	2.01 [c] (±0.62)	1.26 (±0.70)		2.83 [c] (±0.73)	1.37 [c] (±0.82)	1.36 (±0.92)	

[a]95% CI, not significantly different among adjacent values; adjacent values significantly different based on paired t-test for each transect, i.e. submerged vs. 0–1 m above, etc.

[b]95% CI, significantly different among adjacent values.

[c]Paired t-test not significantly different among adjacent values.

when our measurements were taken, the stream width (i.e. wetted width) is only about half the total channel width, as large areas of sand bar are exposed (Fig. 1). At this time, the actual amount of wood surface per square metre of wetted *stream* bottom is 0.380 m^2, rather than 0.191 m^2/m^2 of *channel* bottom (as shown in Table 1). Thus, the amount of wood surface (0.380 m^2) per square metre of *stream* bottom at low water (when stream area is one-half of channel area) is virtually the same as the amount of wood surface (0.379 m^2) per square metre of *stream* bottom at bankfull discharge (when stream area equals channel area) (see total surface area in Table 1). Relatively little sand bar is exposed in the Ogeechee at low water, so the area of wetted stream surface is roughly the same as the area of channel bottom (bank-to-bank).

Total wood surface areas (0.43 m^2/m^2 for Ogeechee and 0.38 m^2/m^2 for Black Creek) (all heights) in each stream are quite high, and are not significantly different. Similarly, total wood volumes at bankfull discharge (0.148 m^3/m^2 for Ogeechee and 0.115 m^3/m^2 for Black Creek) are not significantly different between the streams. When either surface area or volume of wood is expressed per linear metre of stream, the Ogeechee values are about twice those of Black Creek, since the Ogeechee channel is about 50% wider (Table 1). These values are appropriate for channel wood; however, as the river rises above the 2-m height, water begins to invade the flood plain, which greatly increases total wood surface area per metre of stream length.

Mean stem diameter generally decreases from submerged to above-surface heights (Table 1). The diameters are strongly skewed toward smaller sizes by the great abundance of small twigs and branches compared with logs. The decrease in stem diameters is consistent with a general increase in the surface area to volume ratios (square metres per cubic metre), which are 24.7, 24.4, and 53.4 for wood at three elevations in the Ogeechee and 34.6, 28.9, and 98.4 for wood in Black Creek.

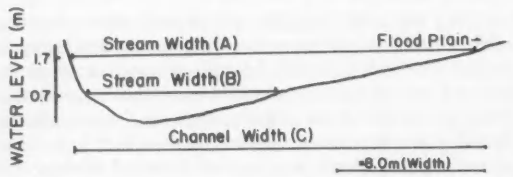

FIG. 1. Schematic illustration of stream width at different water levels compared with channel width for Black Creek. At low hydrograph (stage height = 0.7 m), when wood measurements were made (September 1983), stream width (B) was approximately one-half that of channel width (C). At a stage height of 1.7 m, stream width (A) approximates that of channel width (C).

Although mean stem diameters are strongly skewed toward the smaller stems, larger stems compose the majority of both wood surface area and volume (Table 2). Stems >10 cm in diameter composed over 92% of the total wood volume and 46% of the surface area in the Ogeechee and 74% of the total volume and 35% of the surface area in Black Creek.

Distribution of Wood

Wood distribution within each height (Fig. 2, bottom) can be compared with the depth profile (Fig. 2, top), which indicates the orientation of the wood towards the erosional bank (left side of Fig. 2). One should keep in mind that these are percentages *within* a height, and that absolute volumes between the three heights varied considerably (submerged > 0–1 m > 1–2 m) (Table 1).

All wood distribution is skewed toward the erosional bank; however, the distribution of submerged wood is less strongly skewed than above-surface wood (Fig. 2). Woody material in midstream consists primarily of large tree trunks (some >500 cm in diameter) that have lost most roots and limbs; thus, there is little woody material above the surface in midstream.

TABLE 2. Proportion (%) of total wood surface area and volume contributed by various stem diameter size classes in the Ogeechee River and Black Creek. All channel wood (wetted, 0–1, and 1–2 m above surface) is included.

	Size class (diameter in cm)			
	<1.0	1.0–5.0	5.1–10	>10
Ogeechee River				
% of surface area	8.14	23.77	21.68	46.4
% of wood volume	0.19	1.85	5.15	92.81
Black Creek				
% of surface area	20.99	26.92	17.07	35.02
% of wood volume	0.90	13.11	11.38	74.55

Some of the trunks are >30 m in length and partially buried in the stream bed, which suggests that the total volume of wood in these streams may be greatly underestimated, as buried wood was not measured.

Wood along the erosional bank generally consists of trunks, roots, branches, and twigs of fallen dead trees both above and below the water surface. However, about 25–35% of the above-surface wood consists of live, leaning trees and shrubs that become inundated at higher water. The increase in the 0–1 cm category between 6.7 and 10 m from the bank is attributed primarily to the tops of trees that have fallen into the stream along the erosional bank.

Wood along the depositional bank (right side of Fig. 2) consists primarily of live and dead willows (*Salix* spp.), which tend to grow horizontally from the shore. *Salix* forms valuable substrate for many aquatic insects such as black flies (Simuliidae), chironomids, and net-spinning caddisflies (Hydropsychidae) during periods of high water. Willows also serve as enhanced, although limited, sites for primary production with macrophytes such as *Micranthemum* sp., mosses (*Brachelyma subulatum*), liverworts (*Porella pinnata*), and algae during high water (E. L. Dunn, Georgia Institute of Technology, pers. comm.).

Wood Specific Gravity

Wood (twigs and logs) samples in the Ogeechee and Black Creek had a specific gravity of 0.408 ± 0.028 ($\bar{X}$ ± 95% CI, n = 50). Mean percent ash for wood samples was 1.85 ± 0.38%. Despite their external appearance of advanced stages of decay, logs >10 cm in diameter showed little evidence of any internal decomposition 0.5–1 cm below the surface. Some of these logs were embedded in the stream bed and overlaid with other logs, which suggests they may have been in these streams for many decades. Logs >10 cm in diameter had significantly higher specific gravity ($\bar{X}$ = 0.444 ± 0.028) than limbs and twigs <10 cm in diameter ($\bar{X}$ = 0.379 ± 0.044). The estimated AFDM of wood, based on the product of volume estimates in Table 1 and the specific gravity values of 0.444 (>10 cm in diameter) and 0.379 (<10 cm in diameter), is shown in Table 3. The total wood mass in the Ogeechee is about 6.46 kg AFDM/m^2 of channel bottom vs. 4.98 in Black Creek.

Wood Substrate Surface Area and Discharge

Cumulative wood surface area at water level (submerged) and 1 and 2 m above the water plotted against stream gage height was used to develop predictive equations for estimating wood habitat availability. For the Ogeechee River and Black Creek the curves of best fit were power regressions. For the Ogeechee:

$$(5)\quad S_b = 0.368H^{0.280} \qquad (r^2 = 0.33,\ n = 63)$$

$$(6)\quad S_l = 11.122H^{0.239} \qquad (r^2 = 0.38,\ n = 63)$$

and for Black Creek:

$$(7)\quad S_b = 0.210H^{0.748} \qquad (r^2 = 0.46,\ n = 63)$$

$$(8)\quad S_l = 4.094H^{0.663} \qquad (r^2 = 0.43,\ n = 63)$$

where S_b = square metres of wood surface area per square metre of stream bottom, S_l = square metres of wood surface area per linear metre of stream, and H = stream height (metres). The relatively low r^2 reflects the high variability of wood estimates among transects.

Weekly estimates of submerged snag surface area were made from stream height using equations 5 and 7. Snag surface area follows rather distinct seasonal cycles, increasing during late fall and early winter as water levels rise and decreasing during late spring and summer with falling water levels. Although total discharge varies more than an order of magnitude in the Ogeechee (5–280 m^3/s) and about 3 orders of magnitude in Black Creek (0.05–45 m^3/s) over this time period, in-channel snag surface area varies only threefold (0.15–0.51 m^2/m^2 of channel bottom in the Ogeechee; 0.16–0.48 m^2/m^2 in Black Creek). However, snag surface area is subject to more abrupt changes in Black Creek (Fig. 3).

Equations 5–8 can be utilized to convert animal numbers and biomass into more meaningful ecosystem units. On 19 December 1981, river height was 0.409 m. This value was used in equations 5 and 6 to yield S_b = 0.287 m^2 of wood surface/m^2 of stream channel and S_l = 8.98 m^2 of wood surface area/linear m of stream. The product of these values and the density and biomass of organisms per square metre of snag surface are shown in the last four columns of Table 4. Biomass and density of snag dwelling organisms are still fairly high even when expressed per square metre of channel bottom. The value of 1.88 g dry mass/m^2 of channel bottom for snag animals is considerably higher than all benthic (i.e. non-snag) insects and oligochaetes on this same date (<1 g dry mass/m^2, unpubl. data). When benthic molluscs (1.8 g dry mass/m^2, unpubl. data) are included, total benthic biomass slightly surpasses that of snags.

Discussion

In low-gradient, meandering streams, bank failure along the erosional bank is believed to deliver most woody material to the stream bed (Sigafoos 1964; Keller and Swanson 1979). In the Ogeechee, about 45% of submerged wood is between 3.3 and 10 m (or 11–30% of stream width) from the erosional bank. As these deeply cut banks erode, tree trunks and limbs form rather dense partial debris dams at angles to the main flow. These debris dams are partially stabilized by large roots of fallen trees that remain anchored in the bank. The fallen trees serve to retain other large woody material that may be transported from upstream and the adjacent flood plain forest.

The estimates of wood biomass in our fourth- and sixth-order streams are similar to several first- and second-order streams in other regions, but lower than those from the Pacific Northwest and California redwood forests (Table 5). We should emphasize that Black Creek and the Ogeechee are fourth- and sixth-order streams. We also regard our estimates as low, particularly since

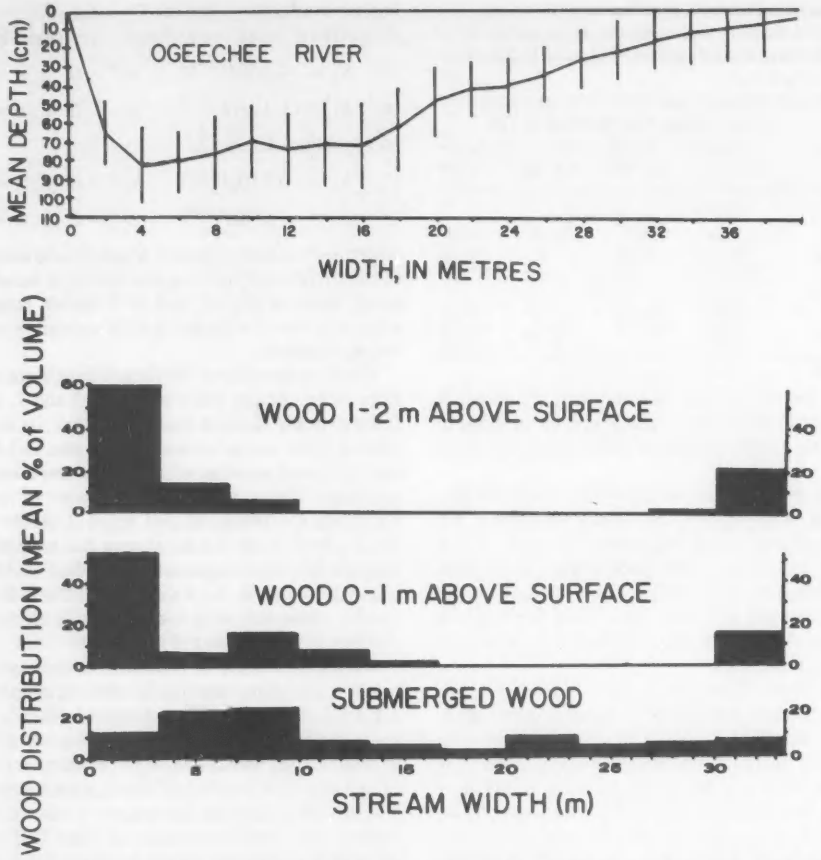

FIG. 2. Top, mean depth profile of the Ogeechee River, with erosional bank at 0 regardless of bank orientation, i.e. east or west bank. The vertical lines represent the 95% CI for depth (cm) at 2-m intervals from the erosional bank during low hydrograph in late August 1983. The apparent width of this depth profile is equal to the width of the longest transect, since each mean depth is based on a fixed distance (up to 40 m) from the erosional bank. Bottom, mean percentage of distribution of wood volume across an "average" transect with a mean channel width of 33 m for wood that is submerged, 0–1 m above surface, and 1–2 m above surface during low hydrograph in late August 1983. These data are based on the percentage *within* each 10% increment of width for each height. Note that wood distribution is skewed toward the erosional bank (see text).

we could not include the very large logs that were embedded in the substrate.

Relationship with Flood Plain

These estimates of snag surface area are only for in-channel wood, and these are valid as systems-level measurements only while the river remains within its banks. At stage height of 2 m (indicated by f.s.O.R. in Fig. 3), total Ogeechee discharge is about 70 m^3/s, with about 18% of discharge flowing through low-lying portions of the flood plain swamp forest. Snag substrate estimates do not consider the unmeasured wood surfaces that become inundated in the swamp. Between the 2- and 3-m heights (f.s., Fig. 3), the river inundates substantial portions of the swamp surface, and these in-channel estimates become increasingly conservative as measures of available habitat surfaces. At the 3-m height, total discharge is 220 m^3/s, with nearly half of total discharge flowing through the swamps, and the amount of swamp surface inundated is more than 10 times greater than the river surface (based on digitized area from infrared satellite photograph, 27 February 1981). In Black Creek on this date, greater than 5 times more swamp surface was inundated than the channel surface area.

Preliminary estimates of woody litter (twigs and logs) on the adjacent flood plain forest floor indicate 1.03–2.3 kg AFDM/m^2 of wood on the Ogeechee flood plain and approximately 0.6 kg/m^2 on the Black Creek flood plain (T. F. Cuffney, University of Georgia, pers. comm.). These latter values are on the lower end of the 0.2–21.8 kg/m^2 range listed by Triska and Cromack (1980) for nine north temperate forests. The estimate of wood in the Ogeechee channel (6.46 kg/m^2) is about 2.8–6.3 times higher than that of the adjacent flood plain forest floor whereas channel wood in Black Creek (4.98 kg/m^2) is 8.3 times greater than wood on its flood plain. This difference may be due in part to movement of wood from the extensive flood plain to the stream during flood stage. Evidence for this is seen on the

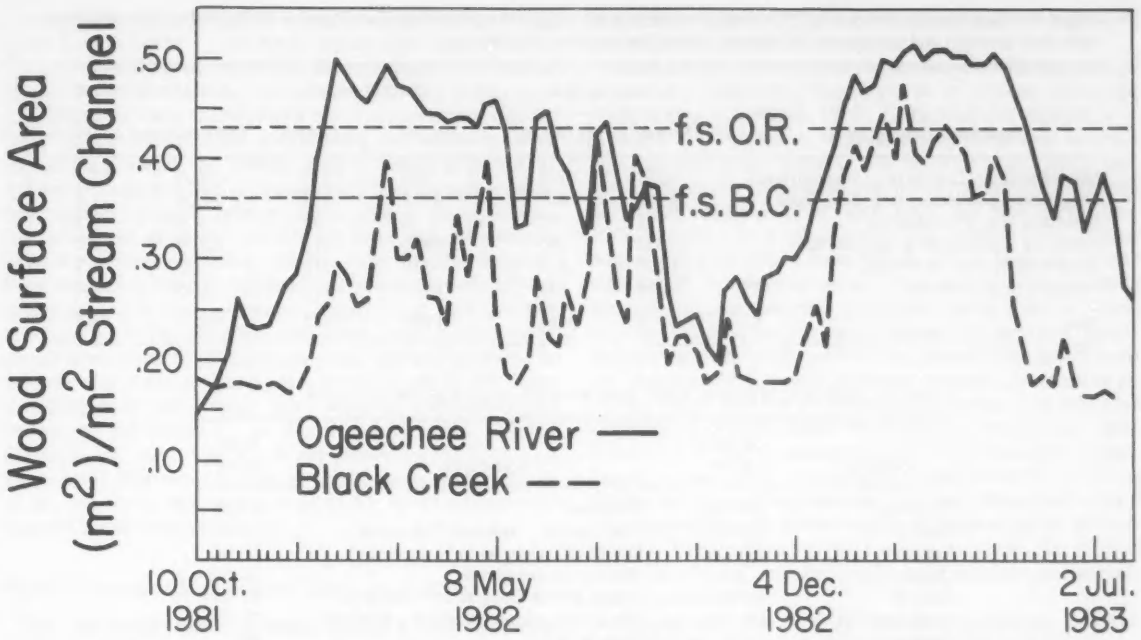

FIG. 3. Weekly means for wood substrate surface area per unit channel bottom for the Ogeechee River (O.R.) and Black Creek (B.C.) during the period encompassing 10 October 1981 through 7 July 1983. These estimates are based on weekly means of stage heights (United States Geological Survey, unpubl. data) regressed with cumulative wood surface area at water level and 1 and 2 m above the surface. These estimates are conservative at stage heights above the level marked flood stage (f.s.) when the streams inundate vast areas of flood plain forest.

TABLE 3. Estimated ash-free dry mass of wood (kg AFDM/m^2 of channel bottom ± 95% CI) in the Ogeechee River and Black Creek for three transect heights in each stream.

	Transect heights			
Stream	Submerged	0–1 m above surface	1–2 m above surface	Total
Ogeechee River	4.407 (±1.83)	1.75 (±0.83)	0.30 (±0.17)	6.46
Black Creek	2.41 (±1.05)	2.45 (±1.60)	0.12 (±0.10)	4.98

flood plain where widely scattered debris masses can be observed, while much of the surrounding forest floor is remarkably clean following floods. Woody litter fall may also be higher along the erosional bank of these streams than in the adjacent flood plain forests. Furthermore, the rate of wood decomposition in water is considerably slower than that on land (e.g. Triska and Cromack 1980; Aumen et al. 1983). Most wood volume in both the Ogeechee and Black Creek is in larger (>10 cm in diameter) stem sizes (Table 2). Since wood decomposition in streams occurs primarily from the periphery toward the interior (Triska and Cromack 1980; Aumen et al. 1983), larger diameter wood decomposes much slower than smaller stems. Hence, the majority of wood volume in the Ogeechee and Black Creek would be expected to have very slow decomposition rates. Thus, entrainment and subsequent retention, higher woody litter input rates, and slower decomposition rates may contribute to higher quantities of wood in these stream channels than on their adjacent flood plains.

Wood distribution in these low-gradient, flood plain river systems is in marked contrast with that of similar stream orders in the Pacific Northwest. In the McKenzie River, Oregon, there is little wood in the river channel (Keller and Swanson 1979; Triska and Cromack 1980; Triska et al. 1982). The McKenzie has a mean annual discharge of 75.3 m^3/s (Naiman and Sedell 1979b), which is only slightly higher than the Ogeechee (67.7 m^3/s). However, calculated as unit stream power, a measure of a stream's ability to perform work (Leopold et al. 1964), where unit power = density × discharge (cubic metres per second) × % slope/width (metres), indicates that the McKenzie has about 35 times more unit stream power than the Ogeechee at mean discharge. This difference is primarily attributable to the much greater slope of the McKenzie (0.6%, Naiman and Sedell 1979b) vs. only 0.02% for the Ogeechee. Unit stream power differences between these rivers increases at high discharge because the Ogeechee widens considerably (flood plain flow) and mean stream velocity declines. Thus, the McKenzie has more capacity to move large materials than the Ogeechee.

Historical Perspective

Early in Georgia history, before the advent of railways, rivers were the main arteries of transportation. Some of the problems of pioneers in navigation of these Coastal Plain rivers are indicated in Fig. 2. Most wood is located along the main thalweg of the river. In 1801, Act 26 of the Georgia Legislature allowed the formation of a company to improve navigation on the Ogeechee River for a distance of about 230 river km. Similar

TABLE 4. Densities and dry mass (DM) of aquatic insects on the snag habitat of the Ogeechee River on 14 December 1981 and their equivalent densities and mass per m^2 of channel bottom and per linear m of stream.

Functional feeding group	Snag surface		Channel bottom		Linear m of stream	
	No./m^2	mg DM/m^2	No./m^2	mg DM/m^2	Thousands/m	g DM/m
Macrofiltering collectors (e.g. *Hydropsyche*)	3 337	4222	958	1212	30.0	37.9
Microfiltering collectors (e.g. *Simulium*)	15 880	827	4 558	237	143.0	7.4
Shredders (e.g. *Pteronarcys*)	55	92	16	26	0.49	0.83
Gathering collectors (e.g. *Stenonema*)	13 332	395	3 826	113	120.0	3.5
Wood feeders (e.g. *Stenelmis*)	1 916	415	550	119	17.2	3.7
Predators (e.g. *Perlesta*)	6 218	611	1 785	175	55.8	5.5
Total	40 738	6562	11 693	1882	366.5	58.8

TABLE 5. Estimates of channel wood mass in various streams in North America.

Location	Stream order	Forest type	Wood (kg/m^2)
California[a]	1st–2nd	Redwood[e]	45–80
Alaska[b]	1st–2nd	Sitka spruce[f] – hemlock[g] (clearcuts)	15.7–61.8
Oregon[a]	1st–2nd	Old growth Douglas fir[h] – hemlock[g]	25–40
Oregon[c]	1st	Old growth Douglas fir[h]	23.6
Oregon[a]	1st–2nd	Young growth Douglas fir[h] – hemlock[g]	20–25
North Carolina[d]	1st–2nd	Old growth mixed hardwood – hemlock[i]	21–24
Oregon[c]	3rd	Douglas fir[h]	14
Alaska[b]	1st–2nd	Old growth Sitka spruce[f] – hemlock[g]	3.2–12.7
Tennessee[a]	1st–2nd	Mixed hardwoods	13
North Carolina[d]	1st–2nd	50- to 60-yr-old mixed hardwoods	10.3
Idaho[a]	1st–2nd	Old growth spruce[j] – lodgepole pine[k]	7.0
Georgia	6th	Ogeechee (this study)	6.5
Oregon[c]	4th	Douglas fir[h] – red alder[l]	5.7
Georgia	4th	Black Creek (this study)	5.0
Michigan[a]	1st–2nd	Young growth mixed hardwoods	4–8
New Hampshire[a]	1st–2nd	Old growth spruce[m] – fir[n]	4
Oregon[d]	7th	Douglas fir[h] – red alder[l] (McKenzie River)	0.75
North Carolina[d]	1st–2nd	15-yr clearcut, all slash removed, burned	<0.5

[a]Triska and Cromack (1980); includes wood > 10 cm; no methods given; dry mass (?).
[b]Swanson et al. (1984); includes all wood diameters using combinations of line intersect and scaling techniques; based on several streams in both clearcut and uncut forests; dry mass.
[c]Naiman and Sedell (1979a); includes all wood; mapping (>10 cm) and handpicking; AFDM (based on 10% ash content).
[d]J. B. Wallace and A. Huryn, unpubl. data; includes all channel wood, using line intersect technique as presented in this study; AFDM.
[e]*Sequoia sempervirens*.
[f]*Picea sitchensis*.
[g]*Tsuga heterophylla*.
[h]*Pseudotsuga taxiflolia*.
[i]*Tsuga canadensis*.
[j]*Picea englemanni*.
[k]*Pinus contorta*.
[l]*Alnus rubra*.
[m]*Picea* SPP.
[n]*Abies balsamea*.

acts were passed the same year for the Savannah, Altamaha, and Oconee rivers. Specifically, these acts called for the removal of as many as possible obstructions (primarily wood) to the navigation of these rivers. Although we have not been able to document the actual amount of wood removed from the Ogeechee, the process was apparently successful (Clayton 1812). By the 1850s many river projects were abandoned in Georgia, especially on smaller rivers such as the Ogeechee where steamboats were impractical, and transportation turned to railways (Coleman et al. 1977). However, the United States Army Corps of Engineers continued to remove snags with snagboats from the Altamaha, Savannah, and lower Satilla rivers until the 1950s (United States Army Corps of Engineers 1953). Thus, snag removal operations have been conducted intermittently in Georgia Coastal Plain rivers over a period of 150 yr. Similar snag removal operations were conducted by the Corps from many rivers throughout the United States during the late 1800s and early 1900s (Sedell et al. 1982).

Snag surface area estimates in both the Ogeechee River and Black Creek (Table 1) are much higher than that made by Benke et al. (1984) for the Satilla River (0.05–0.07 m^2/m^2 of channel bottom) in southeastern Georgia. Two factors may have contributed to some underestimation of snags in the Satilla. First, in the Satilla, only snags along the shoreline were considered. Second, the method used by Benke et al. (1984) differed from the line intersect used here in that horizontal rather than vertical transects (planes) were used at three heights, and submerged wood was not actually measured and assumed to have the same abundance as the mean of other heights. Notwithstanding this possible underestimation, the Satilla River indeed appears to have less woody material than the Ogeechee or Black Creek. The lower wood estimates in the Satilla may be largely attributable to the extensive snag removal operations as recently as the 1940s. During this period about 15 000 snags including trees and stumps were removed from the lower 260 km of the Satilla (J. R. Sedell, U.S. Forest Service, Corvallis, OR, pers. comm.). To our knowledge, there were no other major differences in riparian zone management practices of the two rivers that would account for lower estimates of channel wood in the Satilla.

Potential Impact of Snagging on Coastal Plain Streams

We can assume that man began having a very significant impact on Georgia rivers through snagging efforts initiated by the early 1800s at latest. We predict several potential impacts that may have resulted from early snagging efforts.

First, there was an immediate negative impact on invertebrate diversity and secondary production because of habitat removal. Benke et al. (1984) found that although the snag habitat represented only 6% of the Satilla habitat (compared with muddy backwaters and sand), snags accounted for the greatest diversity of species, and for the river as a whole, over half the invertebrate biomass and 15–16% of secondary production. Preliminary data from the Ogeechee (Table 4) indicate that invertebrate densities and biomass on snags are very similar to those in the Satilla (3–6 g dry mass/m^2 of snag surface). Secondary production of invertebrates on snag substrates in the Satilla River was at least 50 g dry mass$\cdot m^{-2}$ snag surface area$\cdot yr^{-1}$ (Benke et al. 1984). Cudney and Wallace (1980) obtained similar estimates of secondary production on snags in the larger Savannah River. Assuming that Ogeechee snags have somewhat similar levels of secondary production as above (e.g. 50 + g dry mass$\cdot m^{-2}$ snag surface area$\cdot yr^{-1}$) with more snag surfaces the Ogeechee may have 5 times as much snag invertebrate production as the Satilla. Several species of game fishes obtain at least 60% of their food from the snag habitat in the Satilla (Benke et al. 1979; Benke et al., unpubl. data). A snagging operation that removed only 50% of the wood would have a very adverse influence on invertebrate production and the fishes that depend on them for food. Snagging would also remove fish habitat and cover (e.g. Marzolf 1978; Sedell et al. 1982).

Second, the major invertebrate functional group inhabiting snags are filtering collectors that exploit the suspended organic particles made available to them by the current (Cudney and Wallace 1980; Benke et al. 1984). The loss of significant filtering-collector populations from a given reach of stream, combined with losses of snag inhabiting algae and macrophytes, may increase the spiralling length (e.g. Webster 1975; Wallace et al. 1977; Newbold et al. 1982) of nutrients and energy. This would reduce the ability of a given stream segment to process and conserve nutrient and energy inputs.

Third, woody debris is the major item contributing to high roughness coefficients, Manning's n, in streams traversing sandy or clay soils (Chow 1959). In the Manning formula, $V = (1.0/n)R^{2/3}S^{1/2}$, where V = mean velocity (metres per second), R = hydraulic radius (metres), S is the slope of the energy line, and n = Manning's roughness coefficient. Values of n approach 0.150 with the presence of roots, trees, and large logs (Chow 1959, p. 123). This n value exceeds that of fairly clean, regular bottom rivers by over 4 times (Chow 1959). Thus, snags may significantly reduce mean stream velocities and increase settling rates of entrained particles; conversely, snag removal would increase velocity, leading to accelerated transport and thereby further reduce the ability of flora and fauna to process nutrients and organic matter within a stream segment (Swanson et al. 1976). Snags also act as partial debris dams. The increased retention of organic matter may decrease community metabolism, (P/R) ratios, and represent an important factor contributing to the usual heterotrophic condition ($P/R < 1$) of blackwater rivers. Snag removal may also alter river – flood plain interactions via lower roughness, increasing the velocity of flow. This could reduce the frequency and duration of flood plain inundation, and drastically alter nutrient and organic matter pathways.

In summary, the input and retention of woody material, primarily the result of physical processes, provides large, relatively stable substrate for invertebrate colonization in an environment that would otherwise be unsuitable for colonization by many species of animals. The physical environment appears to have influenced the evolution of the biota that exploits these snag substrates, primarily filtering collectors (Cudney and Wallace 1980; Benke et al. 1984), which depend on the entrained particles for their nutrition. As pointed out by Sedell et al. (1982), many state and local agencies emphasize the removal of stream obstructions as a basis for stream and habitat improvement programs. For streams in the southeastern Coastal Plain, the benefits of such operations are, at best, extremely dubious in that snagging operations could seriously reduce both invertebrate and fish production. Similar situations may exist in midwestern streams (based on invertebrate densities reported for woody substrates, Nilson and Larimore 1973) and in the vast lowland streams of the Amazon Basin (e.g. Fittkau et al. 1975; Fittkau 1982; Sioli 1982). Although there are exceptions (e.g. Shields and Nunnally 1984), the best management for such streams and their adjacent flood plains is no "management" other than protection of adjacent flood plains. The biota obviously evolved in response to physical processes long before man interfered with these physical–biological linkages.

Acknowledgments

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Nearshore Ichthyoplankton of a North Temperate Estuary

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Composition of ichthyoplankton in the nearshore zone (0–6 km from shore) of the St. Lawrence estuary showed important differences from that farther offshore. In particular, larvae of herring (*Clupea harengus harengus*), winter flounder (*Pseudopleuronectes americanus*), and smooth flounder (*Liopsetta putnami*) made up a greater proportion of the ichthyoplankton than in published surveys farther offshore. Peak catches of larval herring were 1–2 orders of magnitude higher than in offshore waters. Winter flounder and smooth flounder larvae were significantly more abundant ≤500 m from shore than 1–6 km offshore; significant abundance gradients were not found for other species. Nearshore areas have received considerably less effort than offshore areas in earlier studies, and we suggest that more intensive studies in the nearshore zone, at least for those species mentioned, would be of importance for elucidating early life history processes and for estimating spawning biomass based on ichthyoplankton surveys.

La composition de l'ichtyoplancton de la zone côtière (0–6 km de la côte) de l'estuaire du Saint-Laurent diffère de celle plus au large de façon importante. Les larves du hareng (*Clupea harengus harengus*), de la plie canadienne (*Pseudopleuronectes americanus*) et de la plie lisse (*Liopsetta putnami*) constituent une plus grande proportion de l'ichtyoplancton côtier que dans l'ichtyoplancton plus au large dont la composition est décrite dans des relevés publiés. Les captures maximales des larves du hareng étaient de 1–2 ordres de grandeur plus importantes que dans les eaux plus au large. Les larves de plie canadienne et de plie lisse ont été significativement plus abondantes à des distances ≤500 m de la côte qu'à 1–6 km de la côte ; de tels gradients n'ont pas été démontrés pour les larves d'autres espèces. Moins d'effort ait été dédié aux eaux côtières qu'aux régions plus au large dans des études antérieures et on suggère qu'une intensification des études dans les eaux côtières, au moins pour les espèces mentionnées, pourrait mener à une meilleure compréhension des processus influençant les premiers stades de vie des poissons et à une amélioration des estimés de la biomasse frayante basés sur des relevés d'ichtyoplancton.

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Ichthyoplankton surveys in the past usually have concentrated on open offshore waters accessible to the quantitative sampling gears used on large vessels (Smith and Richardson 1977). The nearshore zone (within approximately 5 km from shore) has, in north temperate waters, been little sampled; thus, its importance for larval fishes is little known. Frank and Leggett (1981, 1983) have conducted a series of intensive studies of ecology of larval capelin and other species in the nearshore zone off Newfoundland, but few other surveys are available. de Lafontaine (1979) described composition of ichthyoplankton on a nearshore transect in the St. Lawrence estuary as well as on station grids offshore. Other studies in north temperature estuaries have described composition and distribution of larval fishes in offshore areas (Jacquaz et al. 1977; Able 1978) or have focussed on ecological processes in deep channels (Graham 1972; Fortier and Leggett 1982; Courtois et al. 1982). Depth is an important variable in the study of larval fish distributions, frequently confounded with distance from shore, and has been treated in several of the studies cited. While most of these earlier studies have touched on distribution and abundance of larval fishes in nearshore areas, comprehensive quantitative information on the spatial distribution of larvae on the inshore–offshore axis over a variety of coastal habitats has not previously been published.

The present study was undertaken to assess the importance of this zone to larval fishes over a stretch of coast showing variations in coastal topography, temperature, and salinity. In particular, if larval fishes were to use the nearshore zone preferentially, data from offshore could be biased in terms of abundance and other ecological measures such as size frequencies, which could bias spawning stock size estimates and lead to erroneous hypotheses on recruitment dynamics. The nearshore zone might be expected to be of importance to larval fishes, since several species of commercial importance move actively into shallow coastal waters (Atlantic herring, *Clupea harengus harengus*; winter flounder, *Pseudopleuronectes americanus*), to beaches (capelin, *Mallotus villosus*), or to rivers (smelt, *Osmerus mordax*) to spawn. Larvae of these species are abundant and larvae of many other north temperate

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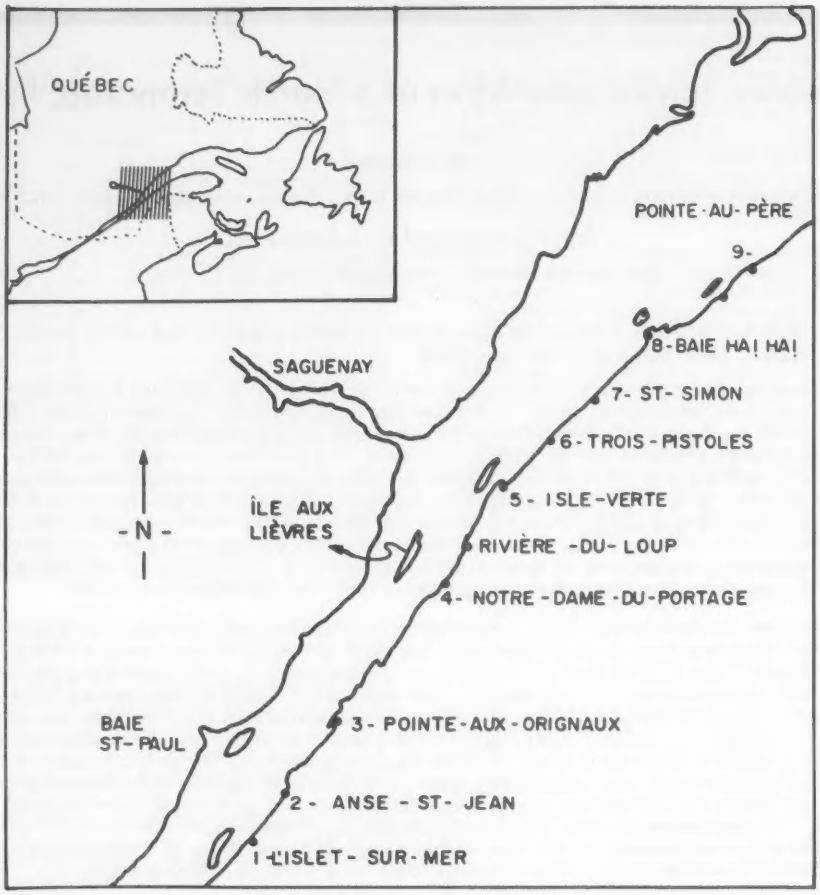

FIG. 1. Location of sampling stations.

species are present offshore in the St. Lawrence estuary (Jacquaz et al. 1977; Able 1978; de Lafontaine 1979). Because of this and because spawning sites are present in the area, we selected it for study.

The objectives of this study were to (1) compare species composition in the nearshore area with that reported from earlier studies offshore in St. Lawrence estuary, (2) determine whether onshore–offshore gradients in abundance exist, at least for the most abundant species, (3) describe spatial and temporal variations in abundance in the nearshore habitat, and (4) relate the observed variations in abundance to physical and chemical characteristics of coastal waters.

Materials and Methods

In 1978, at each of nine stations along the south shore of the St. Lawrence estuary (Fig. 1) plankton tows were made at four distances from shore: 100 m, 500 m, 0.8 km, and 1.6 km. In 1979, six of the nine original stations were sampled; tows were made at six distances from shore: 100 m, 500 m, 0.8 km, 1.6 km, 6.4 km, and 9.6 km. Tows are referred to by capital letters A–F, A being closest to shore. We used a 5.5-m Avon inflatable and a 19.5-m oceanographic vessel. Sampling period was mid-June to the end of November in 1978 and the beginning of May to mid-October in 1979 (Table 1). At each tow site (A–F), a 10-min oblique (bottom to surface) plankton tow was made parallel to the shoreline using a 0.5-m-diameter net with a 0.505-mm mesh and equipped with a General Oceanics flowmeter.

At each tow site, surface temperature was measured in 1978, while surface and bottom temperature and salinity and Secchi depth were recorded in 1979. Salinities were determined either with a Kahlsico induction salinometer or directly in the field with a Beckman salinometer. Environmental conditions at selected stations are summarized in Table 2.

Larvae were preserved in 5% formalin. In the laboratory, larvae were identified and enumerated following subsampling with a Folsom plankton splitter if numbers were large (>300 per sample). For each species, standard lengths of 50 larvae (or all present) were measured to the nearest 0.5 mm with an ocular micrometer and the presence or absence of yolk was noted.

Identifications were made following Jones et al. (1978) for most species, Khan (1971) for Cottidae, and Faber (1976) for blennioid larvae. Identifications of larval Pleuronectidae were verified by W. G. Smith and L. van Guelpen and of larval Liparidae by K. W. Able. All specimens collected are deposited at Huntsman Marine Laboratory, St. Andrews, N.B.

Statistical analyses were performed to examine (1) relative

TABLE 1. Sampling periods in 1978 and 1979.

1978			1979		
Period	Date	Stations	Period	Date	Stations
1	19–29 June	1–9	1	8–18 May	1, 3, 4, 5
2	5–12 July	1–9			7, 9
3	19–27 July	4, 7–9	2	24 May – 6 June	7, 9
4	31 July – 8 August	1–9	3	19–23 June	7, 9
5	5–8 September	1, 3, 5, 7, 9	4	4–13 July	7,9
6	17–25 October	1, 4, 5, 7, 9	5	23–27 July	7, 9
7	9–19 November	1–9	6	6–18 August	7, 9
			7	23 August – 2 September	7, 9
			8	9–16 October	7, 9

TABLE 2. Environmental conditions at selected sampling stations in 1979.

	Temperature (°C)		Salinity (‰)		Depth (m)		Secchi (m)	
Station	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
1, Islet-sur-Mer								
Surface	9.8	23.0	0.1	7.6	—	—	0.1	1.1
Bottom	9.6	22.3	0.2	10.7	4.0	30.0	—	—
3, Pointe-aux-Originaux								
Surface	4.8	13.7	6.1	22.1	—	—	0.5	2.0
Bottom	1.7	10.7	15.5	29.1	4.0	56.0	—	—
4, Notre-Dame-du-Portage								
Surface	5.0	18.5	10.2	24.5	—	—	0.4	3.9
Bottom	2.6	15.2	18.4	29.2	3.0	22.0	—	—
5, Isle-Verte								
Surface	3.7	21.5	16.6	29.5	—	—	0.4	3.9
Bottom	1.6	7.0	23.4	32.3	1.0	100.0	—	—
7, St-Simon								
Surface	4.0	10.9	15.5	29.8	—	—	1.0	5.5
Bottom	1.0	8.4	17.4	33.5	2.0	97.0	—	—
9, Pointe-au-Père								
Surface	4.4	13.6	20.9	29.0	—	—	0.8	6.0
Bottom	0.4	10.4	25.6	33.3	2.0	116.0	—	—

abundance of larvae at different distances from shore, (2) relative abundance between stations, and (3) seasonal abundance. Abundance data (numbers per 10 m^2 surface area) were initially natural logarithm transformed ($\ln X + 1$) and a series of one-way ANOVA's performed to demonstrate abundance differences. A second step was to determine which means were statistically different using Student–Newman–Keuls multiple range tests.

Associations between abundance of larvae of selected species and environmental variables (temperature, salinity, Secchi depth) were investigated using Kendall's coefficient of rank correlation (Sokal and Rohlf 1969).

Results

Species Composition

Species composition of the ichthyoplankton of the nearshore zone is generally similar to that of offshore waters in the St. Lawrence estuary (Able 1978; de Lafontaine 1979), although several marked differences are evident (Table 3). A total of 25 taxa were present in our samples, similar to the numbers taken in earlier studies. Larvae of three species (Atlantic herring, capelin, smelt) dominated the nearshore ichthyoplankton (99.2% in 1978, 96.2% in 1979). Larval herring were the most abundant species in 1978, capelin in 1979. Larval herring made up a higher proportion of the nearshore ichthyoplankton than that ofshore (Able 1978; de Lafontaine 1979), probably because of the presence of a herring spawning site near Isle-Verte (Able 1978).

Larval pleuronectids ranked fourth (smooth flounder) and fifth (winter flounder) in abundance in 1979 (Table 3), although these species were relatively rare in earlier studies. Larvae of these species are more abundant nearshore than offshore (see below), which probably accounts for their greater relative importance. The absence of smooth flounder larvae in the 1978 samples is probably due to lack of sampling in May 1978, which appears to be their spawning time.

Larvae taken during the present study but absent from earlier

TABLE 3. Fish species captured along the south shore of the St. Lawrence estuary in 1978 and 1979.

Species	Sources[a]	1978 Total no.	1978 %	1979 Total no.	1979 %
Clupeidae					
Clupea harengus	1, 2	142 543	86.6	52 463	37.0
Alosa sapidissima	1, 2	—	—	8	<0.1
Osmeridae					
Mallotus villosus	1, 2	17 018	10.3	75 737	53.5
Osmerus mordax	1	3 805	2.3	8 028	5.7
Gadidae					
Enchelyopus cimbrius	1, 2	2	<0.1	—	—
Gadus morhua	1, 2	—	—	1	<0.1
Microgadus tomcod	1	—	—	30	<0.1
Scorpaenidae					
Sebastes sp.	1, 2	1	<0.1	—	—
Cottidae					
Gymnocanthus tricuspis	1, 2	—	—	7	<0.1
Myoxocephalus aeneus	1, 2	16	<0.1	—	<0.1
Cottus sp.		2	<0.1	—	—
Agonidae					
Aspidophoroides monopterygius	1, 2	—	—	15	<0.1
Cyclopteridae					
Cyclopterus lumpus	1, 2	236	0.1	13	<0.1
Liparis altanticus	1, 2	390	0.2	345	0.2
Liparis gibbus	1, 2	—	—	85	<0.1
Stichaeidae					
Ulvaria subbifurcata	1, 2	383	0.2	290	0.2
Stichaeus punctatus	2	—	—	8	<0.1
Pholidae					
Pholis gunnellus	1, 2	2	<0.1	122	<0.1
Ammodytidae					
Ammodytes sp.	1, 2	35	<0.1	946	0.7
Catostomidae					
Catostomus sp.		—	—	21	<0.1
Pleuronectidae					
Pseudopleuronectes americana	2	106	<0.1	973	0.7
Liopsetta putnami	2	1	<0.1	2 443	1.7
Percichthyidae					
Morone americana		—	—	3	<0.1
Percidae					
Perca flavescens		—	—	50	<0.1
Gasterosteidae					
Gasterosteus sp.		—	—	1	<0.1
Total		164 540	100.0	141 623	100.0

[a]1, Able (1978); 2, de Lafontaine (1979).

studies offshore are species of high estuarine or freshwater distribution (*Catostomus* sp., *Morone americana*, *Cottus* sp., *Perca flavescens*, *Gasterosteus* sp.). The almost complete absence of gasterosteid larvae from the nearshore samples is remarkable given that Gasterosteidae are abundant in salt marshes along the south shore of the St. Lawrence estuary (FitzGerald 1983). Larvae of the four species present would be expected to occur from the end of April to the end of July. The absence of larvae from coastal waters may indicate that they remain in tide pools and streams where spawning occurs.

Species absent from our collections, but noted in the offshore ichthyoplankton studies cited, are those of the open sea or low estuary areas (e.g. Atlantic mackerel, *Scomber scombrus*; longhorn sculpin, *Myoxocephalus octodecemspinosus*; Atlantic halibut, *Hippoglossus hippoglossus*). Two such species may spawn too early to have been taken by us; de Lafontaine (1979)

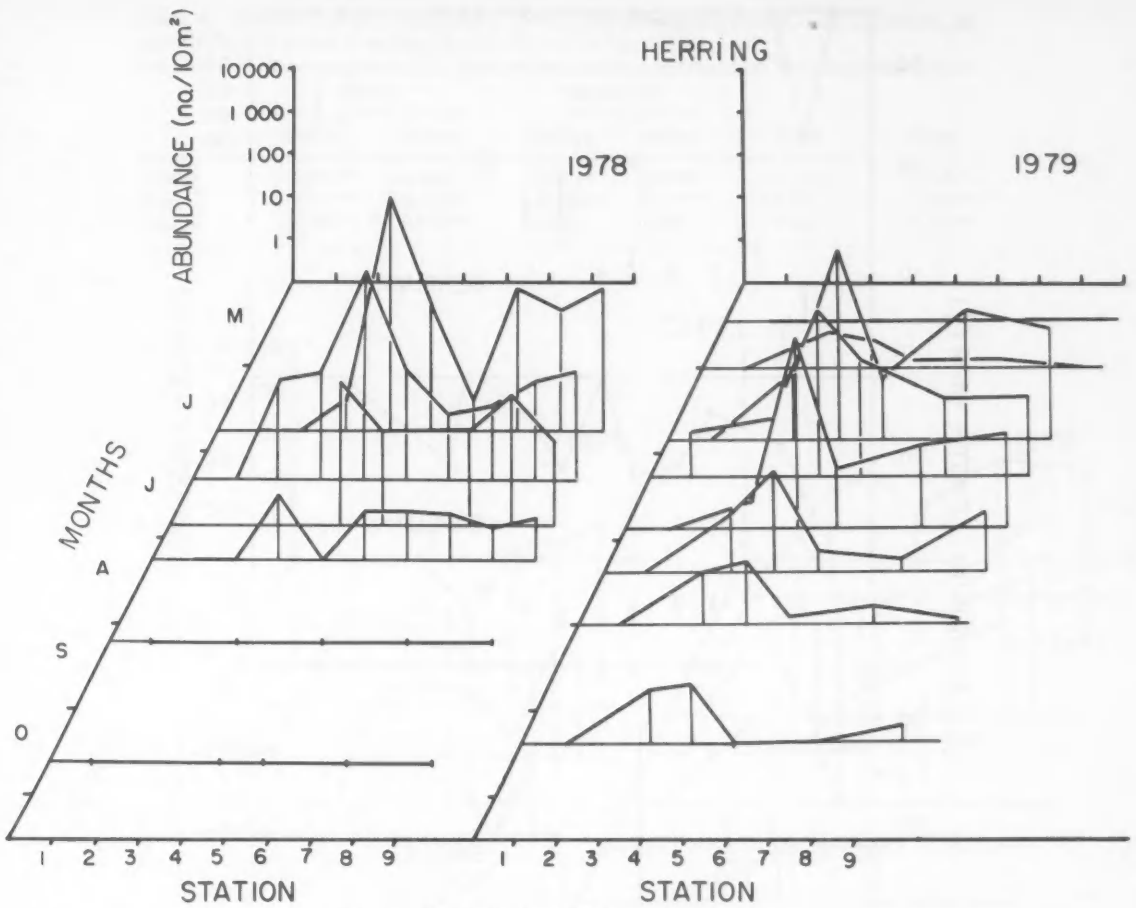

FIG. 2. Abundance and distribution of larval herring.

noted the presence of daubed shanny (*Lumpenus maculatus*) and shorthorn sculpin (*Myoxocephalus scorpius*) only at the end of April.

Distribution and Growth of the Most Abundant Species

Herring

Herring larvae were present at some time of the sampling period at all stations, the greatest abundance being observed at Notre-Dame-du-Portage during June and July (Fig. 2). One-way ANOVA revealed no significant differences in mean abundance of larvae at different distances from shore, so in subsequent tests all tows at each station were considered as replicates.

In 1978, there were differences in abundance among stations for the first and second sampling period (ANOVA, $p < 0.001$) and for period 4 (ANOVA, $p < 0.05$). In 1979, there were differences in abundance among stations for periods 3–8 (mid-June to mid-October) (ANOVA, $p < 0.01$). In both years, herring were more abundant at Notre-Dame-du-Portage (station 4) than elsewhere (Student–Newman–Keuls, $p < 0.05$) and there were no other significant differences among stations.

The abundance and size of larvae at Notre-Dame-du-Portage (station 4) with the presence of yolk sac larvae in June and July (Fig. 3) indicates that sampling was probably conducted close to a spawning bed. Following the peak in larval abundance in June–July, abundance dropped dramatically (Fig. 2). In 1978, larvae were rare or absent at most stations by September. In 1979, larvae remained relatively numerous at stations 3 and 4 until sampling was terminated at the end of October.

Larvae of similar sizes (Fig. 3) were common in the coastal zone from late May to late July (including the period of maximum abundance) followed by a steady increase in length to approximately 25 mm by mid-October. Two periods of abundant yolk sac larvae (late May, early July) were observed (Fig. 3).

Significant but weak positive correlations were observed between larval herring abundance and surface salinity, surface and bottom temperature, and Secchi disk depth (Table 4). A significant, weak negative correlation was observed with bottom depth. These relationships are contradictory, since temperature and salinity vary inversely from upper to lower estuary, while Secchi disk depth generally parallels salinity (Table 2). The relationship with depth may be explained by the occurrence of maximum abundance at Notre-Dame-du-Portage where depths over the transect sampled were generally shallow (Table 2).

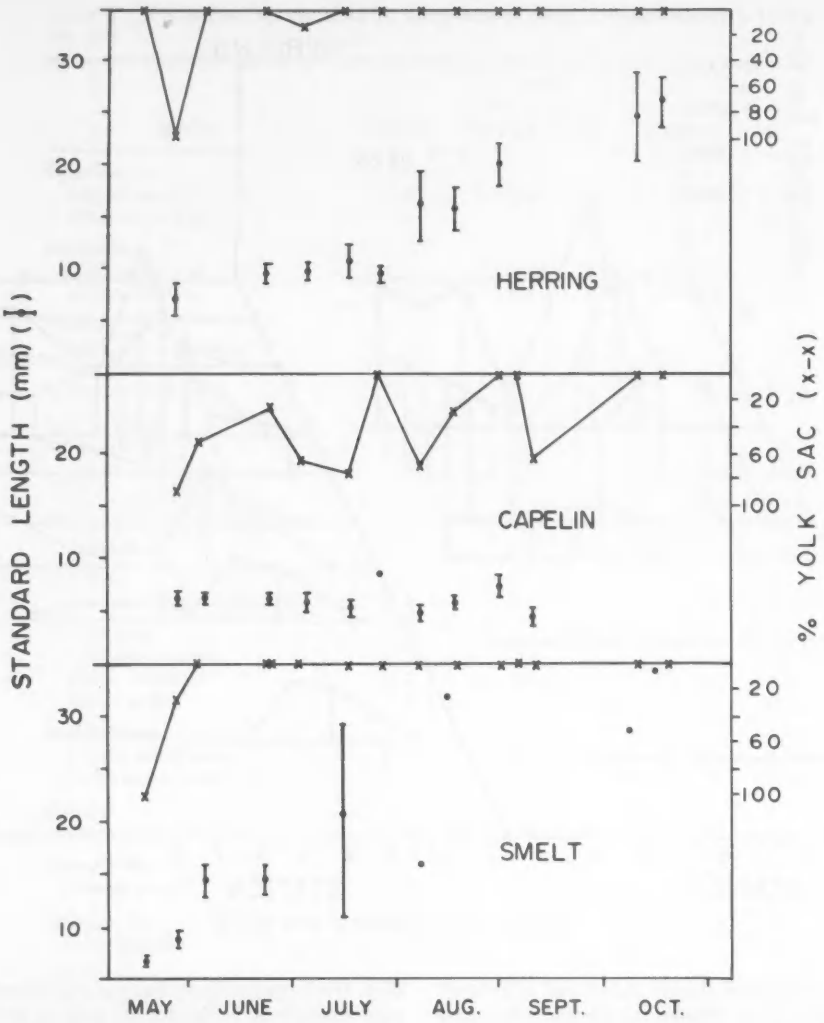

FIG. 3. Seasonal changes in percent yolk sac larvae and standard lengths (±1 SD) for herring, capelin, and smelt, 1979.

In summary, it would appear that hatching of herring eggs is concentrated temporally in June and early July and spatially off North-Dame-du-Portage. Larvae remain for some period of time in the coastal zone after hatching (since abundance remains very high for 1–2 mo, lengths remain low, but yolk sacs are absent from most larvae) but disperse offshore in August and September (since abundance in the coastal area decreases dramatically and length increases). Although the beginning of the period when larval herring were present corresponded with that noted by Tremblay (1942) off Isle-Verte, we found herring larvae to be present over a longer period than he did. Tremblay found no herring larvae after 5 July, while our study showed larvae present in the coastal area to early August (1978) or October (1979). The up-estuary movement of larval herring with growth noted by Able (1978) is confirmed by our observation of some large larvae at stations 3 and 4 in September and October.

Capelin

As with herring larvae, one-way ANOVA revealed no significant differences in abundance with distance from shore, so tows at each station were treated as replicates and were pooled. Larvae were at maximum abundance in late June throughout most of the survey area; in 1979 when sampling was conducted earlier than in 1978, hatching apparently began in early June, since larval abundance was very low in late May (Fig. 4). Larvae remained in the coastal zone in appreciable numbers until September.

Larval abundance generally increased from upstream to downstream (Fig. 4). In 1978 there were differences in abundance between stations during periods 1, 2 ($p < 0.001$), and 4 ($p < 0.05$) (mid-June to early August), while in 1979, differences occurred among stations in all periods except October ($p < 0.01$); however, no station consistently had maximum abundance.

TABLE 4. Kendall's correlation coefficients between larval abundance of herring, smelt, and capelin and selected physical–chemical variables in 1979 (*$p < 0.05$; **$p < 0.01$).

	Salinity		Temperature			
	Surface	Bottom	Surface	Bottom	Depth	Secchi
Herring	0.126**	−0.604	0.091*	0.229**	−0.152**	0.140*
Smelt	−0.437**	−0.416**	0.381**	0.319**	−0.019	−0.408**
Capelin	0.189**	0.189**	−0.027	0.009	−0.019	0.312**

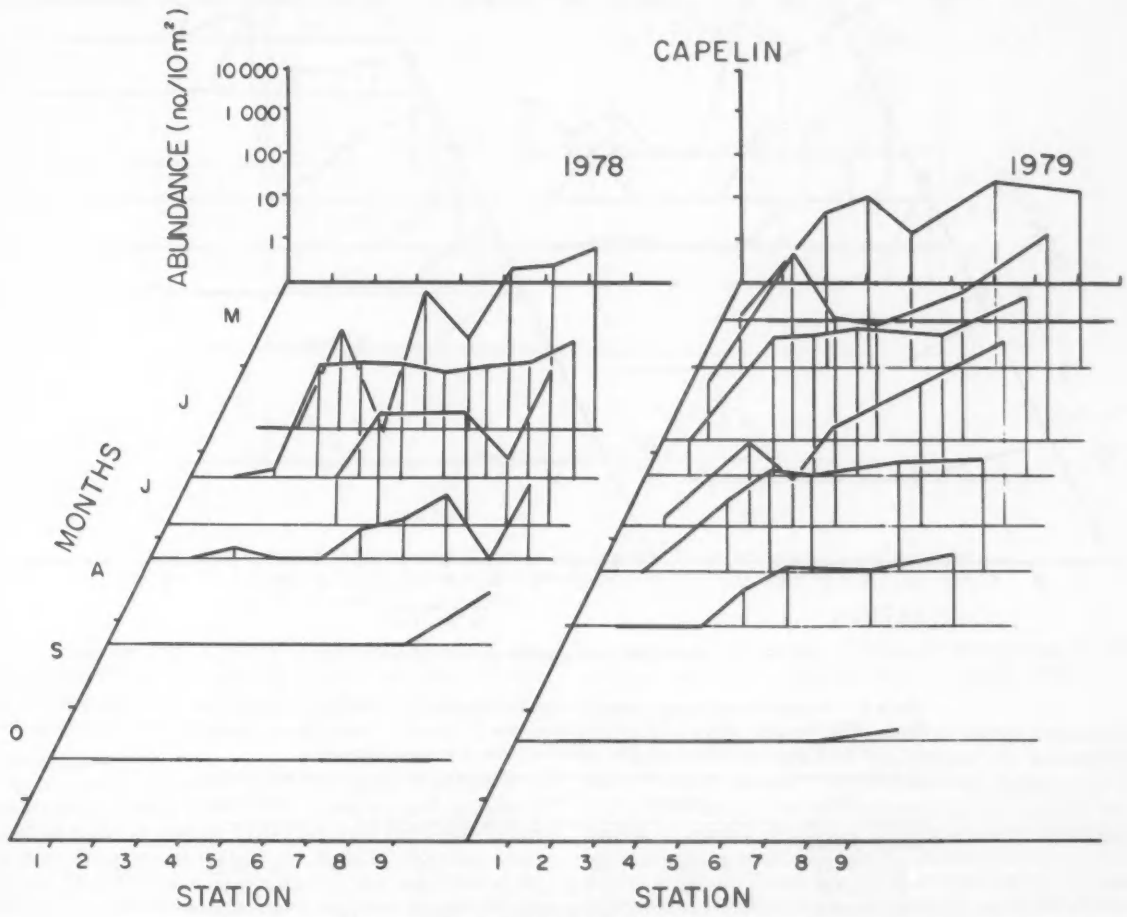

FIG. 4. Abundance and distribution of larval capelin.

The consistently small size of larvae and the occurrence of yolk sac larvae during most of the sampling season (Fig. 3) show that hatching occurs from June to the beginning of September and that dispersal out of the sampling area is rapid and constant. Jacquaz et al. (1977) obtained similar results in offshore waters of the St. Lawrence estuary and concluded that larvae were rapidly flushed from the estuary to a retention area in a gyre west of Anticosti. The proportion of yolk sac larvae was variable over the sampling period in 1979 (Fig. 3), which may result from cyclical or occasional release of larvae into the coastal habitat (as observed by Frank and Leggett 1981). Capelin abundance was positively correlated with salinity and Secchi disk depth (Table 4), consistent with the spatial pattern of greater abundance in the low estuary than in the high estuary.

Smelt

As with herring and capelin larvae, no significant inshore–offshore gradients in abundance were detected, so tows at each station were pooled. In both 1978 and 1979, abundance was different between stations from the start of sampling (mid-June in 1978, early May in 1979) to early September (1978: $p < 0.001$ for periods 1, 2, 4, $p < 0.05$ for period 5; 1979: $p < 0.001$ for periods 1–4 and 6, $p < 0.01$ (period 7). In all cases, the differences were due to significantly greater abundance

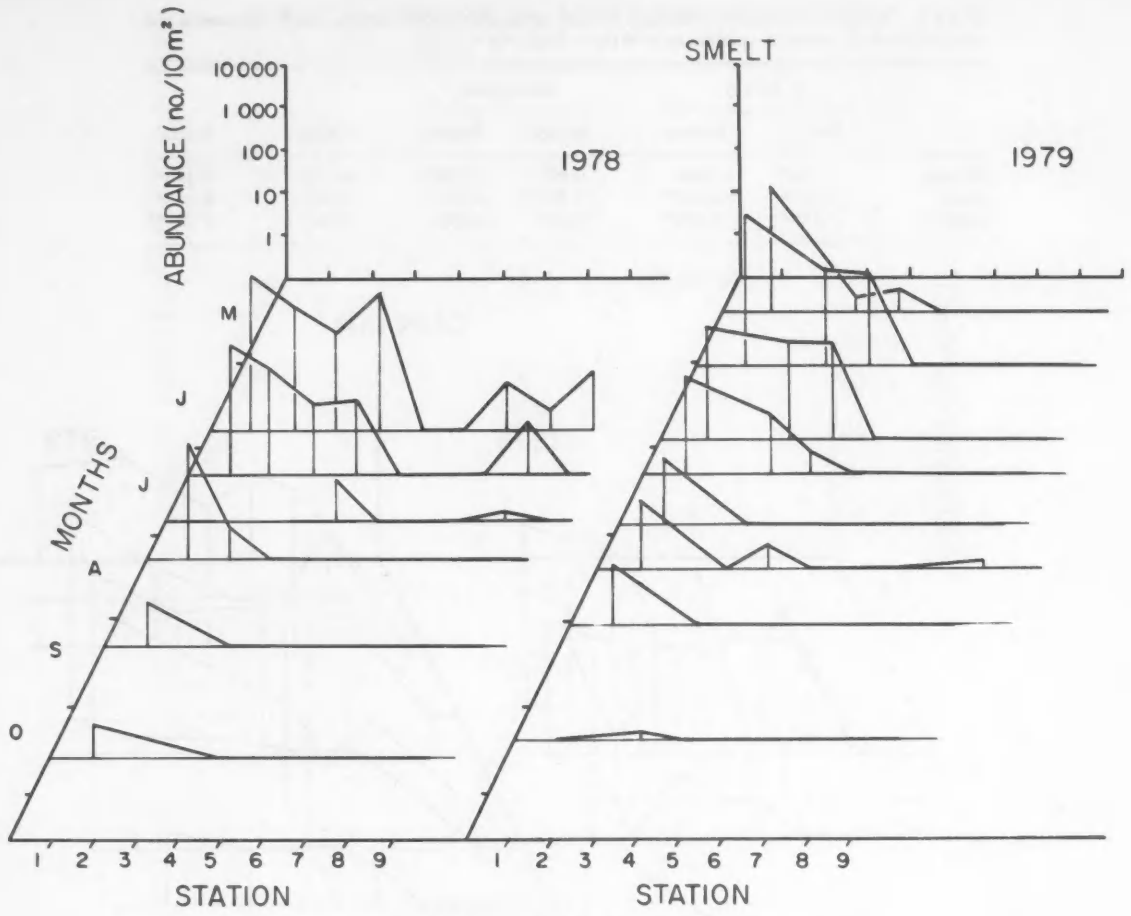

FIG. 5. Abundance and distribution of larval smelt.

TABLE 5. Results of ANOVA for inshore–offshore differences in abundance of larval flatfishes, 1979. Tows A + B are <0.5 km from shore; tow C–F are 0.8–6 km offshore. Stations 4 + 5 (Notre-Dame-du-Portage and Isle-Verte) are those of greatest abundance.

Date	Stations	Tows	F	df	Significance
Smooth flounder					
16–18 May	All	All	1.62	5,30	$p < 0.25$
16–18 May	All	A + B vs. C–F	7.87	1,34	$p < 0.01$
16–18 May	4 + 5	A + B vs. C–F	24.02	1,10	$p < 0.001$
28 May – 6 June	All	A + B vs. C–F	4.88	1,34	$p < 0.05$
Winter flounder					
8 May – 6 June	All	A + B vs. C–F	3.54	1,70	$p < 0.10$
8 May – 6 June	4 + 5	A + B vs. C–F	6.63	1,22	$p < 0.005$
19 June – 6 August	All	A + B vs. C–F	0.68	1,94	$p < 0.5$

at station 1 (L'Islet-sur-Mer) than at any other station. Species composition of l'Islet-sur-Mer was different than at other stations, including several freshwater species not taken elsewhere. A turbidity maximum and temperature–salinity front separate this station from those farther down estuary (d'Anglejan and Ingram 1976). Larvae were abundant throughout the sampling period at station 1 but were found only early in the season (May–July) at other stations. Abundance of larvae generally declined from station 1 (up estuary) to station 9, and rarely were significant numbers of smelt larvae taken downstream from station 4 (Notre-Dame-du-Portage) (Fig. 5). Yolk sac larvae were not observed after the end of May in the coastal zone and mean lengths increased steadily from May to October (Fig. 3). Abundance of larval smelt was positively correlated

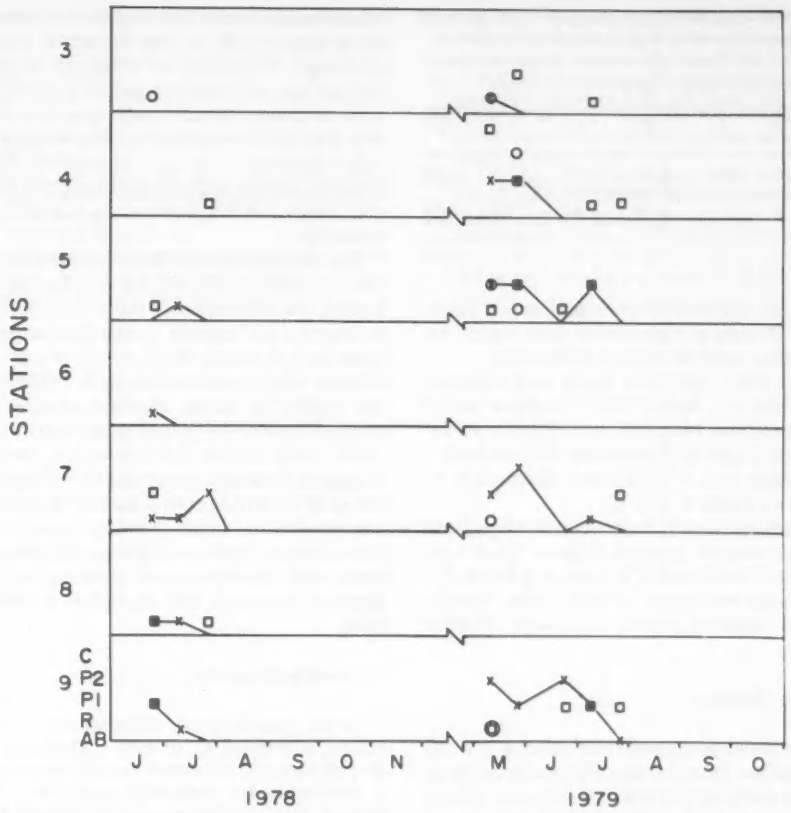

FIG. 6. Abundance and distribution of larval smooth flounder (○), winter flounder (□), and sand lance (×—×) (average no./10 m^2, all tows at station date). Abundance levels (no./10 m^2): AB, 0; R, 0.1–0.9; P1, 1.0–9.9; P2, 10.0–49.9; C, 50.0–149.9.

with temperature and negatively correlated with salinity and Secchi depth (Table 4). These relationships are consistent with the spatial pattern of greater smelt abundance in the high estuary than in lower estuary areas.

Smooth flounder

Larval smooth flounder were only taken at distances of <0.8 km from shore (tows A–C). Larvae were significantly more abundant at distances <0.5 km from shore than farther offshore (Table 5), at stations and dates of maximum abundance. This difference in abundance was not revealed by ANOVA on all stations and tows together, but showed up clearly when mean abundance at tows A and B (<0.5 m offshore) was compared with that at tows C–F (0.8–6 km) (Table 5).

Because of the offshore–onshore gradient in abundance, abundance values at stations were not pooled to test for between-station differences. However, smooth flounder larvae were most abundant at stations 4 and 5 and were rarely taken at other stations (Fig. 6).

Smooth flounder larvae were only taken in May and early June, the latest capture being June 25 1978 and 6 June 1979 (Fig. 6). Maximum abundance was recorded in early May at station 4. Larvae were 5–6 mm long throughout the period when larvae were captured. However, a considerable range of developmental stages was present, from early post-yolk sac to post-caudal flexion larvae; considerable development of this species is undergone in this size interval (Laroche 1980).

Winter flounder

Winter flounder larvae were also more abundant nearshore than farther offshore; however, this gradient was less marked than for smooth flounder. The difference was significant ($p < 0.005$) when the analysis was performed on data from the two stations of greatest abundance (stations 4 and 5) during the period of greatest abundance (8 May – 6 June) (Table 5). For all stations combined during this period and later in the year, the difference in abundance between nearshore and offshore tows was not significant ($p < 0.10$) (Table 5). Although ANOVA's on ichthyoplankton data are not very revealing when numbers are low, it appears from the F-values that larvae may disperse offshore later in the season as development advances (Table 5).

Winter flounder larvae were taken at all stations except stations 1 and 6 and throughout the period May–July (Fig. 6). Peak abundance values were in May – early June at stations 3, 4, 5, while later in the season larvae were taken over the entire region sampled. Maximum length of larvae taken was 4.2 mm.

Thus, the general distribution pattern of winter flounder larvae appears to be concentrated in the nearshore zone during peak abundance and a more dispersed pattern later in the season; the change may be due to dispersal of larvae with development or to variation in spawning areas with season.

TABLE 6. Distribution of larval sand lance along the south shore of the St. Lawrence estuary (stations 1–9). Significance level for Student–Newman–Keuls test is 0.05. Lines join stations where abundance values (ln X + 1 transformed) are not significantly different.

Date	1	3	4	5	7	9
8–18 May	0	0.48	0.71	0.79	1.68	2.85
28 May – 5 June	0	0	1.30	1.54	2.64	2.19

Sand lance

ANOVA revealed no onshore–offshore gradients in abundance, so data from all tows at each station were pooled for examination of between-station abundance differences.

During both periods when sand lance larvae were abundant (8–18 May and 28 May – 5 June 1979), abundance varied significantly between stations (ANOVA, $p < 0.001$). Abundance was significantly higher at downstream stations than at upstream stations, suggesting a "maritime" distribution of larvae within the estuary (Table 6; Fig. 6).

Larvae were most abundant early in the season (May–June) but were taken to early August. Lengths of larvae taken were essentially constant at 5.5–6.0 mm. Constant length coupled with declining abundance with time, as with winter flounder larvae, resembles the "capelin pattern" of constant flushing from the hatching area.

Discussion and Conclusion

The nearshore zone has received much less attention in north temperate ichthyoplankton studies than offshore areas; however, the results of this study suggest that more intensive studies in the coastal zone could throw light on early life history processes of species spawning nearshore. The studies of Frank and Leggett (1981, 1983) demonstrate the extent to which small-scale intensive studies nearshore can contribute to the understanding of early life history processes. Their study concentrated on capelin, but our results suggest that early life history processes of herring and pleuronectids should be studied in the inshore zone.

de Lafontaine (1979) sampled a coastal transect off Rimouski and noted that winter flounder larvae were taken only on the two inshore stations (depths 0–10 m), a finding that our study confirms over a wider area. On the other hand, our study did not confirm an observation of Marcotte and Tremblay (1948) to the effect that larval smelt are found only near shore. Those authors noted that smelt larvae were absent from samples taken more than 1 mile (1.6 km) from shore. Since the sampling intensity on which this observation was based was not given, it is possible that this was limited to a small area. The marked spatial structuring of larval distributions noted in our study corresponds closely to the observations of Courtois et al. (1982) who noted strong spatial separation of the three most abundant species of larval fishes in the St. Lawrence estuary. Inshore–offshore abundance gradients characterize distributions of marine fish larvae in coastal waters off Hawaii over approximately the same spatial scale as studied here (Leis 1982).

Estimates of spawning biomass from larval surveys might be improved by greater concentration on the nearshore area. Nearshore surveys are inexpensive relative to large-vessel surveys offshore, and in areas such as on station 4, where larval herring are abundant, young larvae may be concentrated in relatively small areas. Concentration of surveys on the youngest larval stages (yolk sac and immediate post-yolk sac) has the advantage of reducing net avoidance to the minimum, since reaction and swimming capacities generally increase with size in larval fishes. A preliminary survey for a spawning biomass estimate of St. Lawrence estuary hering (Auger and Powles 1980) indicated that such surveys are feasible logistically; however, further study of small-scale processes (tidal transport, patchiness) was found necessary to improve larval abundance estimates.

The mechanisms contributing to the fine spatial structuring of ichthyoplankton such as observed here are still incompletely known, but information is available from several recent studies to describe and explain spatial habitat partitioning in larval fishes (e.g. Courtois et al. 1982). It may be questionable to attribute such spatial structuring to "habitat partioning," since this implies an active selection of areas, and larval fishes probably have only a limited capacity in this regard. Henri et al. (1985) have shown that interactions between physical and biological processes can account for a high degree of aggregation of larval herring even in the very earliest larval stages (in the area in which maximum abundance values were observed in the present study). Spatial structuring of ichthyoplankton appears to result from a combination of spawning site selection by adults, physical processes, and biological adaptations of larvae to these.

Acknowledgments

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Relative Importance of Anthropogenic versus Natural Sources of Acidity in Lakes and Streams of Central Ontario

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LaZerte, B. D., and P. J. Dillon. 1984. Relative importance of anthropogenic versus natural sources of acidity in lakes and streams of central Ontario. Can. J. Fish. Aquat. Sci. 41: 1664–1677.

Anthropogenic acid deposition has been implicated in the acidification of surface waters of central Ontario and elsewhere. However, alternative sources of acidity have been proposed, including (i) the release of protons and their accompanying acid anions by soils (e.g. organic acids) and (ii) the exchange of internally generated protons for the cations of atmospherically deposited neutral salts (e.g. sea salts). We show that for a typical acidified catchment in central Ontario the first hypothesis is not important during periods of high discharge or on an annual basis. As most of the neutral salts deposited on inland areas are sulphate and nitrate of anthropogenic origin, any acidity generated via the second mechanism can be reduced by reducing the emission of the salts. A third and alternative hypothesis is that deposition of strong acid alters only the balance between humic anions and sulphate in runoff waters, but not pH; we have shown this to be incorrect using catchments from the Sudbury region of Ontario where rates of acid deposition have recently declined. We conclude that in central Ontario, acidic deposition derived from anthropogenic SO_2 sources is the primary causal factor in recent lake acidification, and that a reduction in the anthropogenic emissions affecting this area will result in a decrease in lake acidity.

Les dépôts acides d'origine anthropogène sont tenus responsables de l'acidification des eaux superficielles de l'Ontario et d'ailleurs. Toutefois, il y a d'autres sources d'acidification, notamment : (i) la libération de protons et de leurs anions acides correspondants par les sols (les sols acides) et (ii) l'échange de protons produits intérieurement contre des cations de sels neutres déposés par l'atmosphère (sels marins). Nous montrons que pour un bassin acidifié typique du centre de l'Ontario la première hypothèse n'a que peu d'importance pendant les périodes de forte décharge ou sur une base annuelle. Comme la plupart des sels neutres déposés à l'intérieur des terres sont des sulfates et des nitrates d'origine anthropogène, toute acidité produite par le second mécanisme pourrait être réduit en diminuant les émissions de sels. Une troisième hypothèse possible serait que le dépôt d'acides forte modifie seulement l'équilibre entre les anions humiques et les sulfates dans les eaux de ruissellement, mais pas le pH ; nous avons montré, en utilisant des bassins de la région de Sudbury, en Ontario, où les dépôts acides avaient récemment baissé, que ce n'était pas le cas. Nous en avons donc conclu que dans le centre de l'Ontario les dépôts acides provenant de sources anthropogènes de SO_2 étaient la cause principale de l'acidification récente des lacs, et qu'une réduction de ces dépôts pourraient entraîner une diminution de l'acidité des lacs.

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The deposition of strong acids of anthropogenic origin onto soils and vegetation overlying the Precambrian Shield of central Ontario, and onto other sensitive areas in North America and Europe, is implicated in the acidification of the dilute waters draining these soils (Henriksen 1980; Harvey et al. 1981; Overrein et al. 1981; Dickson 1983; Dillon 1983). However, alternative sources of the acidity in streams and lakes have been proposed (Rosenqvist et al. 1980; Patrick et al. 1981; Nilsson 1983; Krug and Frink 1983), including (i) the release of protons and their corresponding acid anions from soils and vegetation (e.g. organic acids) and (ii) the exchange of protons by soils and vegetation for the cations of neutral salts deposited from the atmosphere (e.g. sea salts). A third hypothesis was recently suggested by Krug and Frink (1983); they have acknowledged the current importance of strong acid deposition to the acidification of certain lakes and streams, but have argued that a reduction in strong acid deposition will induce the release of soil *humic* acids in nearly equivalent amounts with "little or no measurable change in pH."

The first alternative hypothesis is based, in part, on observations that natural acids are normally involved in the release and transport of cations (Johnson et al. 1972; Likens et al. 1977; Cronan et al. 1978). For example, in well-drained mineral soils, fulvic acid anions transport iron and aluminum from the A to the B horizon before they precipitate in the process of podzolization. The bicarbonate anion of carbonic acid migrates farther into the streams draining the soil (Cole and Johnson 1977; Ugolini et al. 1977; Johnson 1981; McDowell 1982) carrying with it any basic cations released by cation exchange or mineral dissolution. In waterlogged organic soils, the fulvic acid anions also migrate into open waters (e.g. Cronan et al. 1978; Hemond 1980; Dawson et al. 1981) before precipitation.

Without these "mobile anions" (Cole and Johnson 1977), any cations derived from acid weathering (or any residual protons) could not migrate out of the soils. Although the natural acids involved in these processes are primarily carbonic and organic acids, another possible source includes sulphuric acid from the oxidation of reduced sulphur.

The importance of organic acids to the acidity of ombrotrophic sphagnum swamp waters has been emphasized by Hemond (1980), and Dawson et al. (1981) have documented the leaching of fulvic acids from water-saturated forest soils (see also Cronan et al. 1978). The ubiquitous presence of dystrophic waters on the Canadian Shield (Armstrong and Schindler 1971; P. J. Dillon, unpubl. data) and in the Maritime provinces of Canada (Kerekes 1980) suggests that organic acids derived from waterlogged organic soils may be important contributors to the acidity of freshwater catchments in regions subject to high levels of acid deposition and elsewhere.

The second alternative hypothesis (Rosenqvist 1978) is based, in part, on observed accumulations of *nonmobile* organic acids that naturally acidify northern soils and sphagnum swamp soils (Gorham and Cragg 1960; Clymo 1963; Rosenqvist 1978; Hemond 1980). These acids, although immobilized, can also acidify water passing around them by proton exchange in which cations (e.g. Ca^{2+} or NH_4^+) are removed from solution and replaced with an equivalent number of free protons. More importantly, the biological uptake of cations, incompletely balanced by the uptake of anions, can also induce soil acidification (Rosenqvist 1978; Ogner 1980; Nilsson et al. 1982) by the release of protons or assimilation of hydroxyls needed to maintain charge balance. Consequently, a higher proportion of protons on the soil's cation exchange sites results, reducing "base saturation." The differential uptake of NH_4^+ relative to NO_3^- is the primary source of these protons (e.g. Matzner and Ulrich 1983). With direct biological uptake, as with proton exchange by immobilized humic and fulvic acids, there is no release of acid anions; these "mobile" anions must be obtained from the neutral salts (e.g. sea salts) in atmospheric deposition (Nilsson et al. 1982; Miller 1983) for the acidity to be exported to streams and lakes.

Rosenqvist (1978) and Nilsson (1983) have argued that recent increases in soil acidification (e.g. because of changes in land use) could be causing the increased acidification of surface waters, particularly in maritime environments where there can be large quantities of sea salt deposition. Consequently, a reduction in deposition of anthropogenic strong acids would have little impact (however, see Nilsson et al. 1982 and Christophersen et al. 1982).

In central Ontario and nearby regions of North America, however, sea salts are negligible and anthropogenic sources dominate the total deposition of strong acid anions (Galloway and Whelpdale 1980). Consequently, even if these anions were somehow neutralized before deposition, and then completely reacidified by "natural" processes, the internal generation of acidity could still be eliminated by eliminating the deposition of the anthropogenic anions. Of course, most strong acid anion deposition in central Ontario and nearby regions of North America is not neutralized anyway, and when it is, the cation is usually NH_4^+, which has an acidifying effect on the environment as well (Galloway and Dillon 1983). We conclude that if the natural acidification of deposited neutral salts in central Ontario is a problem, it can be controlled by reducing their deposition.

Thus, for the remainder of this paper we will only consider the first alternative hypothesis concerning the internal release of protons and their accompanying acid anions by soils (e.g. organic acids) and the third alternative hypothesis (Krug and Frink 1983) that strong acid deposition alters the balance between humic anions and sulphate in runoff waters, but not the pH.

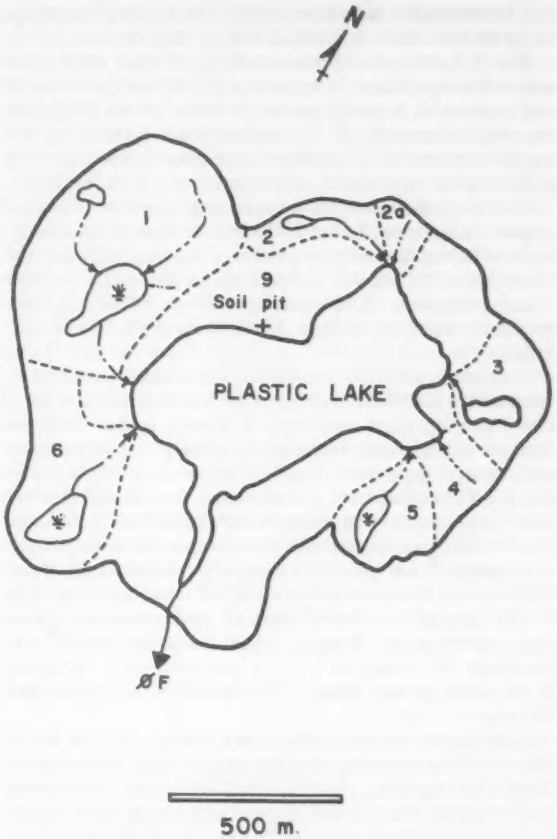


FIG. 1. Subcatchments of Plastic Lake, Ontario, with their major drainage paths (Nos. 1, 2, 2a, 3, 4, 5, 6), the soil pit (No. 9), and the outflow (OF) indicated.

Selection and Description of Study Sites

We chose Plastic Lake catchment to quantify the relative importance of organic acids because fishkills during spring snowmelt have occurred in this lake and have been attributed to the input of strong acids of anthropogenic origin (Harvey and Lee 1982).

The Plastic Lake catchment (area 88.6 ha) is located in Haliburton County, Ontario. The catchment is primarily underlain by ortho-gneiss and covered by very thin surficial deposits (<1 m thick), bedrock ridges, and organic soils. There are five inflowing streams (Fig. 1) that drain sphagnum–conifer swamps (Nos. 1, 2, 5, 6) or a beaver pond (No. 3), of which only No. 1 flows in late summer; the others flow only in the spring and fall. In addition, there are two soil–water seeps (Nos. 2a, 4) not draining any obvious ponds or depressions that also flow only in the spring and fall. A soil drainage pit (No. 9)

was located along the northwest shoreline to monitor seepage along the soil–bedrock interface directly into the lake.

The third alternative hypothesis (Krug and Frink 1983) is that a reduction in strong acid deposition will induce the release of soil humic acids in nearly equivalent amounts with "little or no measurable change in pH." To evaluate this hypothesis, we first apply it to spring runoff in Plastic Lake, assuming a reduction in anthropogenic depositions, and critically evaluate the results. We also test it more directly by examining the effects of reduced sulphur emissions at Sudbury, Ontario, on the average acidity, organic carbon, and sulphur content of nearby Clearwater and Swan lakes. The bedrock and surficial geology of the Sudbury lakes' catchments (Clearwater and Swan lakes) has been previously described in detail (Dillon et al. 1982; Jeffries et al. 1984).

In addition, we refute several lines of evidence provided by Krug and Frink (1983) in support of their hypothesis. One of their more important supporting arguments is that "acid rain does not increase cation leaching in strongly acid soils at rates equivalent to deposition. Rates of cation export from watersheds in the northeastern United States, Canada and Norway which are thought to be sensitive to acid rain are remarkably low." To evaluate this argument we compare the annual cation export rates of two typical catchments in central Ontario (Harp and Dickie lakes) where deposition of strong acid averages 70–100 $meq \cdot m^{-2} \cdot yr^{-1}$ with those of the Rawson lake catchment (northwestern Ontario), which has a low rate of acid deposition (8–10 $meq \cdot m^{-2} \cdot yr^{-1}$), and with those measured in the Sudbury area where acid deposition has approached 200 $meq \cdot m^{-2} \cdot yr^{-1}$.

Dickie Lake's catchment (Muskoka District, Ontario) has an area of 450 ha excluding the lake surface and is underlain by hornblende migmitite. Approximately 80% of the catchment is covered by thin till (<1 m thick) and rock ridges, with slightly thicker mineral till deposits (5%), and organic deposits (15%) covering the remainder (Jeffries and Snyder 1983).

The Harp Lake catchment has an area of 509 ha and is underlain by gneiss and other metasedimentary bedrock including amphibolite and schist. Approximately 50% of the catchment is covered by a minor till plain (sandy loam and sand deposits between 1 and 3 m thick), with thin deposits (<1 m thick) and rock ridges covering most of the remainder. Some very thick deposits (>3 m) occur in some of the valleys. The till is often covered by organic deposits in the stream valleys.

The soils in the well-drained parts of catchments of both Dickie and Harp lakes can be classed as brunisols with small areas of podzols having a poorly defined Ae horizon. The cation-exchange capacities (ammonium acetate extraction) of the A and B horizons average about 60 and 30 meq/100 g, respectively, with base saturation of only about 5 and 2%, respectively. The thick deposits (>3 m) in the bottoms of the valleys of the Harp Lake catchment have C horizons with cation-exchange capacities of about 10 meq/100 g with base saturation of about 5%, but these soils cover a very small portion of the total catchment. Organic matter has accumulated in areas saturated with water for a large part of the year.

Overburden in the Rawson Lake catchment is generally less than 1 m deep, with bedrock (principally granidiorite) outcrops on ridges and valley slopes (Schindler et al. 1976). Brunskill and Schindler (1971) classified these soils as brunisols. Thicker deposits (2–15 m) in the low-lying areas are composed of silicate sand with some calcite and dolomite. The groundwater chemistry was reported to exhibit properties of a combined silicate–carbonate weathering system.

Methods

In order to evaluate the importance of organic acids relative to mineral acids entering Plastic Lake, we calculated the relative loading of different anions to Plastic Lake during the spring (and summer) of 1982. The emphasis was placed on spring snowmelt because of Harvey and Lee's (1982) observations on fish kills during this period, but also because in Plastic Lake, as in many other lakes and streams believed to be affected by anthropogenic acids, the lowest pH's are recorded at this time (Jeffries et al. 1979; LaZerte 1984), and the volume of water entering and potentially acidifying the lake is at its greatest (see Results).

By assuming that all anions originally enter the catchment in acid form and that their measured abundance was equivalent to the number of protons dissociated, we could estimate their relative contribution to the acidification of the upstream catchment and to the free acidity entering the lake. This approach was possible because the congruent dissolution of primary carbonates and sulphates from the soils and bedrock in this area, as in most other regions sensitive to acid deposition, is negligible (Likens et al. 1977; Cole and Johnson 1977; Jeffries and Snyder 1983).

The discharge of the major inflow (No. 1) to Plastic Lake, which drains 27% of the lake's catchment area, was continuously monitored with a V-notch weir (Scheider et al. 1983). The discharge of all other inflows into the lake was estimated by multiplying the measured discharge of the main inflow by the ratio of the area of the minor inflow to that of the major inflow. The areas of the subcatchments were determined by field survey with an estimated accuracy of ±5% or better.

Over the period of our study (spring, 1982), surface runoff from approximately 41% of the catchment area was directly monitored chemically, and an additional 39% of the catchment area without surface runoff was estimated from the soil pit (No. 9) chemistry. The remaining 20% of unmonitored area (primarily subcatchment No. 6) was estimated from adjacent monitored subcatchments. Sampling of all chemical parameters was performed twice weekly during the spring of 1982 (25 February to 17 May) or as long as the streams were flowing sufficiently to collect the samples. For some of the soil water seeps (e.g. No. 2a) this was only 4–6 wk.

The discharge of the five major inflows to Dickie Lake and the four major inflows to Harp Lake was continuously monitored using V-notch weirs, rectangular weirs, or flumes (Scheider et al. 1983) beginning in June 1976. The output of substances from each subcatchment was measured as the product of the stream discharge and the concentration of the substances in the stream measured 102–160 times per stream over the 48-mo period from June 1976 to May 1980. Sampling frequency was greatest during periods of high discharge. Input of substances from the atmosphere was measured by bulk deposition (Scheider et al. 1979). Net export was calculated as the total (gross) export less the input as bulk deposition.

In the Sudbury area, Clearwater Lake was sampled between 6 and 23 times per year from 1973 to 1983, whereas Swan Lake was sampled 19 times in 1977 and 11 times in 1982 (Ontario Ministry of the Environment 1982a).

Sulphate and chloride were measured by ion chromatography beginning in 1980. Prior to this, sulphate was measured in the

Sudbury lakes and precipitation by the methylthymol blue method. Comparison of the two methods indicated that in these exceptionally clear acid waters there was no measurable difference between the methods. Nitrate was measured by the hydrazine sulphate method, ammonia by the phenol-hypochlorite method. Calcium, Mg, Mn, Fe, Na, and K were analyzed by flame atomic absorption; pH was measured with a glass electrode standardized against NBS buffers; dissolved inorganic carbon (DIC) was analyzed colorimetrically by automated dialysis against an acid phenolpthalein solution (Ontario Ministry of the Environment 1981). Bicarbonate concentration was computed from DIC and pH using the extended Debye–Huckel theory to estimate activity coefficients and the dissociation constants of Harned and Davis (1943). Organic carbon was estimated by a digestion procedure that adequately digests dissolved organic acids, but only slightly digests particulate carbon (Ontario Ministry of the Environment 1981). The samples were filtered with a 76-μm mesh Nitex filter to remove leaf litter, if present, and the samples were also allowed to settle before subsampling for the analysis. For these reasons, the organic carbon measured in this manner is called "dissolved" organic carbon (DOC; Ontario Ministry of the Environment 1981), although the usual 0.45-μm filtration step is eliminated. Organic aluminum, free and total fluoride, and the various forms of inorganic monomeric aluminum were estimated as described by LaZerte (1984).

The concentration of organic anions in solution was estimated in two ways: by charge balance and with an empirical model (Oliver et al. 1983). A comparison of the two methods (see Appendix) showed that they were approximately equivalent, and thus the simpler empirical model was used in the actual anion budget calculations.

Results

Sources of Acidity to Plastic Lake, Spring–Summer 1982

Using the model of Oliver et al. (1983) we calculated the proportion of different anions that have contributed free protons. These data and the hydrologic discharge for the major inflow to Plastic Lake for spring snowmelt, 1982, and for the summer months (18 May to 25 August) are presented in Fig. 2. The sulphate anion dominates the anion chemistry during peak flow (Fig. 2), accounting for 83% of the total proton contributing anions; but during low flow in the summer months, sulphate levels abruptly drop. This is the result of seasonal anoxia in an upstream sphagnum–conifer swamp during low flows (B. D. LaZerte, unpubl. data) and is characteristic of many streams in this area draining such swamps (P. J. Dillon, unpubl. data). The organics are the second largest source of acidity in this system, but at peak flows they represent only 8–13% of the total proton contributing anions. However, later in the summer, during periods of low discharge, they can dominate. Although nitrate is usually unimportant in this stream, an early spring peak was detected before the high spring flow. Field observers (B. Locke and B. Girard, Ontario Ministry of the Environment, Dorset Research Centre, Dorset, Ont., pers. comm.) noted that this was the result of an early spring rain (14 March 1982) which followed ice channels in the snow pack and was diverted directly to the streams with little or no contact with the soils. The anion chemistry of this rain was dominated by nitrate (79 μeq NO_3^-/L), as well as sulphate (91 μeq SO_4^{2-}/L).

Similar chemistry was found in another stream draining a sphagnum–conifer swamp (Fig. 3a) and a stream draining a beaver pond (Fig. 3b). However, the beaver pond outflow was usually anaerobic and characterized by readily detectable (by odor) levels of H_2S. The increasing levels of bicarbonate (as seen in the "other" anion category of Fig. 3b) may be the result of a positive H_2S interference with the DIC method (Ontario Ministry of the Environment 1981). In contrast with the above streams, soil water seeps were clear, ephemeral, and overwhelmingly dominated by the sulphate anion (Fig. 3c) during their period of flow. Since this study these trends have been verified at other soil water seeps in the Plastic Lake catchment, some of which flow well into June (B. D. LaZerte, unpubl. data). The soil pit site (Fig. 3d) was somewhat more colored during early snowmelt, apparently because it had just been dug out and the site was still disturbed. As more water flowed through it, however, it became very clear and dominated by sulphate.

Organic acids make up a very small proportion of the total anions supplied to the lake (Fig. 4) during spring melt. In addition, the snowmelt directly off the lake's surface is not included in this loading estimate and the lake represents 27% of the total catchment area; as organic acids are negligible in the snowpack, the relative importance of organic acid loading to the lake is reduced even further. As the snowpack does contain nitrate, however, our estimate of nitrate loading should be considered a minimum.

Effect of Reduced Sulphur Emissions on Sudbury Lakes

The emissions of sulphur dioxide from the ore smelters at Sudbury, Ontario, have declined from an average of 1.41×10^6 metric tons (t)/yr in 1973–77 to 0.67×10^6 t/yr in 1978–82 (Table 1), a drop of approximately 54% (Ontario Ministry of the Environment 1982b). The levels measured in 1973–77, in turn, represent a substantial reduction from the 2.20×10^6 t/yr emitted in the preceeding two decades (1953–72).

Between 1973 and 1977, the sulphate concentration in Clearwater Lake averaged 545 μeq/L. Following the drop in sulphur emission levels in 1978, the sulphate concentration in Clearwater Lake began to decrease (Table 1). By 1983, the mean concentration was 379 μeq/L (a drop of approximately 30%) and appeared to be still declining. Since the water replenishment time of the lake averages 3–4 yr (Scheider 1984), changes in concentration may be expected to continue for some time. Also, the sulphate residence time in the soils is almost certainly greater than the water residence time in the soils (e.g. Christophersen and Wright 1981). In fact, the high sulphate concentrations measured in 1973 likely reflect the sulphur emission rates that occurred prior to 1973.

As the sulphate concentration of Clearwater Lake has decreased, the pH of the lake has increased. From a mean pH of 4.23 (calculated from the arithmetic mean of H^+ concentration) in 1973–77, the lake pH had risen to 4.60 by 1983. This represents a change in the free hydrogen activity of 57%, from 59 to 25 μeq/L, even greater than the change in sulphate. There has been no corresponding change in the organic content of Clearwater Lake. DOC has remained extremely low, averaging 0.4 mg C/L in 1981–83 compared with 0.6 mg C/L in 1973. Water color also has not changed, averaging <5 Hazen units at all times. Applying the Oliver et al. (1983) model (see Appendix), these organic carbon levels indicate that there was

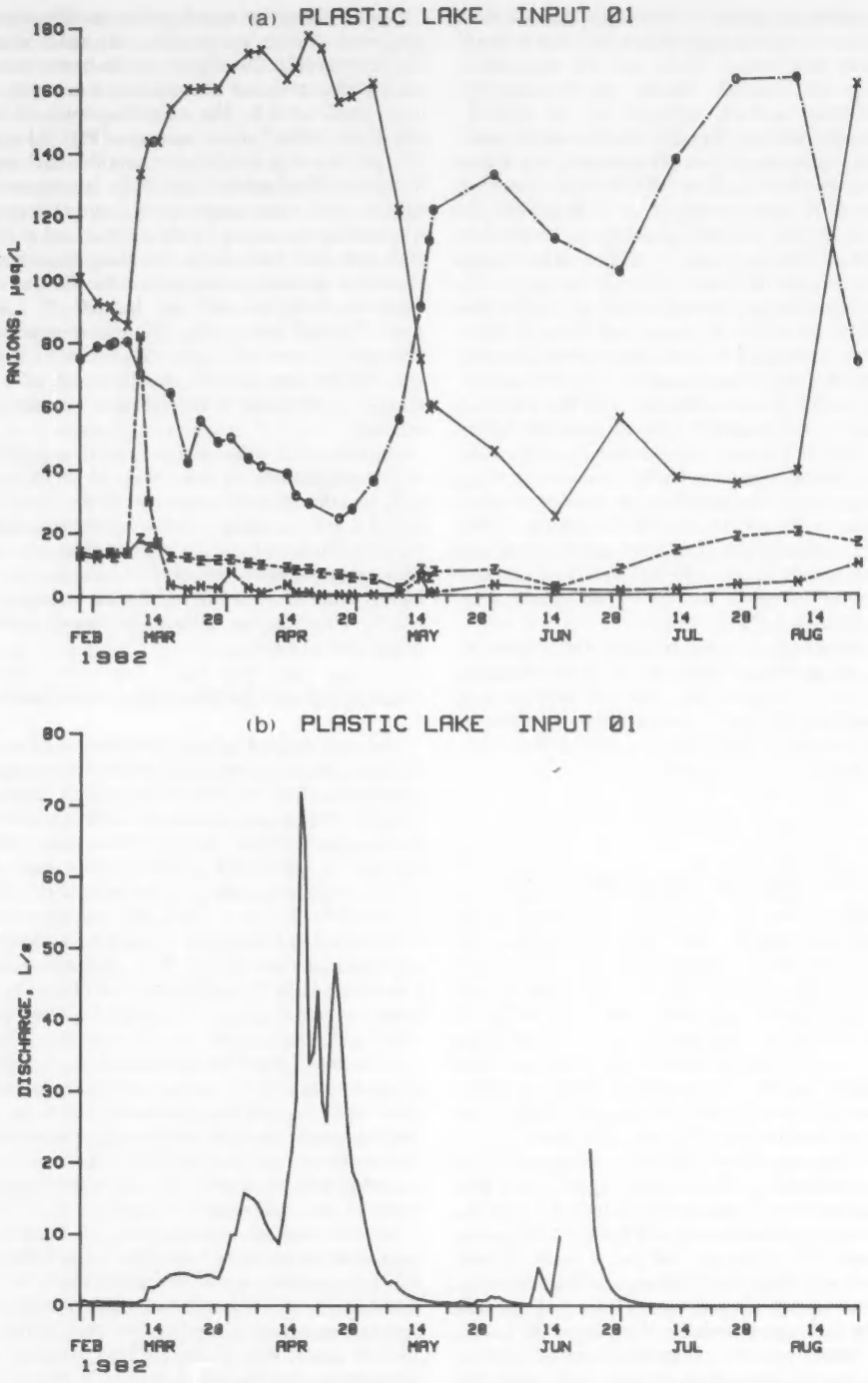


FIG. 2. (a) Anion equivalents for sulphate (*X*), organic anions (O), nitrate (N), and other, primarily chloride and bicarbonate (Z), in the main stream draining the Plastic Lake catchment; lines are connecting the points for convenience; (b) discharge of the same stream.

only 2–3 μeq dissociated organic acid functional groups/L in Clearwater Lake at any time, a level far too low to have any appreciable effect on lake pH.

Swan Lake was studied less intensively. In 1977, its pH averaged 3.96 (110 μeq H^+/L, $N = 12$) and the sulphate concentration was 580 μeq/L. By 1982, the sulphate levels had dropped 62% to 220 μeq/L and pH had increased to 4.80 (16 μeq H^+/L, $N = 12$), an 85% decrease in H^+ activity. As with Clearwater Lake, DOC remained low and there were no measurable changes in DOC or color. The larger decreases in the sulphate and proton concentrations of Swan Lake relative to Clearwater Lake may be the result of Swan Lake's lower water replenishment time (about 1 yr; W. A. Scheider, Ontario Ministry of the Environment, Rexdale, Ont., unpubl. data).

The change in sulphate concentration in the lakes is not expected to be proportional to the change in local emissions, since the deposition of sulphur from the atmosphere is not directly proportional to local emission rate. It is well documented that a significant proportion, perhaps as much as 50%, of the sulphur deposited in the Sudbury area in 1978–79 originated from outside the Sudbury region (Scheider et al. 1981; Ontario Ministry of the Environment 1982b). The overall change in Clearwater Lake's sulphate concentration may, once new steady-state concentrations are reached, remain substantially less than the change in emissions (54%). The greater proportional decrease in sulphate concentration in Swan Lake is likely a result of sulphate reduction and precipitation in the increasingly anaerobic hypolimnion between 1977 and 1982.

Effect of Strong Acid Deposition on Cation Export

Strong acids deposited on Precambrian catchments with no carbonate component in the bedrock or surficial till may be neutralized by incongruent dissolution of silicates or by adsorption–exchange on the surface of minerals or amorphous materials. The result is the exchange of protons from deposition for the basic cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) from the catchment. Comparison of cation export (or yield) from areas with different deposition rates of strong acid provides information on the relative importance of this mechanism of proton assimilation.

Cation export from catchments has been measured in three areas of Ontario that receive very different rates of strong acid deposition. The deposition rates near Sudbury averaged about 200 $meq \cdot m^{-2} \cdot yr^{-1}$ during the course of the studies reported here (Ontario Ministry of the Environment 1982a), whereas those near Dorset averaged 70–100 $meq \cdot m^{-2} \cdot yr^{-1}$ (Scheider et al. 1979; W. A. Scheider, unpubl. data), and those at Rawson Lake (Experimental Lakes Area) in northwestern Ontario averaged 8–10 $meq \cdot m^{-2} \cdot yr^{-1}$ (Schindler et al. 1976).

Net cation flux was lowest at the site with the lowest deposition of strong acids (Rawson Lake; Table 2). This cation flux is, however, greater than the deposition rate of strong acids alone, indicating that natural processes including carbonic and organic reactions also control cation export (D. W. Schindler, Freshwater Institute, Winnipeg, Man., pers. comm.). The importance of carbonic acid reactions is supported by the observed output of bicarbonate (about 14 $meq \cdot m^{-2} \cdot yr^{-1}$) relative to the cation flux.

The cation flux at Dorset was 2–3.5 times greater than that at Rawson Lake, while the strong acid deposition was an order of magnitude greater. Bicarbonate flux from carbonic acid weathering was substantially less than the cation flux and much less than the strong acid deposition. The cation fluxes were greater in the Harp Lake catchment than in the Dickie Lake catchment, which is consistent with the fact that the former has thicker overburden.

The cation fluxes in the Clearwater Lake catchment near Sudbury were the highest despite the fact that its overburden was the thinnest of all the reported sites. The bicarbonate flux (2 $meq \cdot m^{-2} \cdot yr^{-1}$) was negligible; a considerable portion of the strong acid deposition was not neutralized, explaining the difference between deposition and basic cation flux.

Discussion

Is the "Natural" Deposition of Acid Anions Important?

In the introduction we cited two possible sources of "natural" acidity to surface runoff. One involves the "natural" deposition of strong acid anions which, if neutralized before deposition, are reacidified by proton exchange. We argued that this mechanism is unimportant in central Ontario as naturally derived strong acid anions make up a small proportion of the total in deposition.

Is the Internal Release of Acid Anions Important?

A potentially more important source of natural acidity for inland ecosystems was considered to be the internal release of acids, including the acid anion. Our results, however, demonstrate that on an annual basis organic acids do not contribute significantly to the acidity of Plastic Lake catchment; rather, the results show that sulphuric acid is the primary source. Although organics can dominate in some streams during low-flow periods in summer, their impact on the lake during periods of high flow, as during spring snowmelt, is low, and consequently their overall effect is small.

Is the strong acid anion, sulphate, derived from natural internal sources or from anthropogenic deposition? Clearly, from Fig. 2 there exist internal sinks and sources of sulphate that operate on a seasonal basis (see also Christophersen and Wright 1981 for similar results in Norway), so the important question is whether there are long-term primary sources of sulphuric acid derived from the weathering of ancient pyrite. Studies of unweathered surficial bedrock mineralogy in this area indicate that little or no pyrite is present (less than the detection limit of 0.02% S w/w, Jeffries and Snyder 1983); these low levels are certainly insufficient to export the quantities of sulphate we observed. This is also generally conceded for other areas in North America affected by acid deposition (Likens et al. 1977; Galloway et al. 1983; Krug and Frink 1983), implying that atmospheric deposition is the primary source of sulphur. As there are, however, possible local exceptions (e.g. Gorham 1958; Galloway et al. 1983), detailed long-term sulphate budgets are required to test this assertion rigorously for catchments in central Ontario. Such budgets are currently being compiled and preliminary calculations suggest that the assertion is supported (P. J. Dillon, unpubl. data).

We conclude that the major source of acidity in Plastic Lake catchment is anthropogenically derived sulphuric acid obtained from atmospheric deposition. This conclusion is in agreement with the results of Cronan et al. (1978) for soil water leachates in a New Hampshire forest, but extends their results to a large, whole lake catchment in central Ontario. Although we have only presented data here for one catchment, we consider it representative of what might be expected in other nearby sensitive areas. This expectation is being tested over a range of

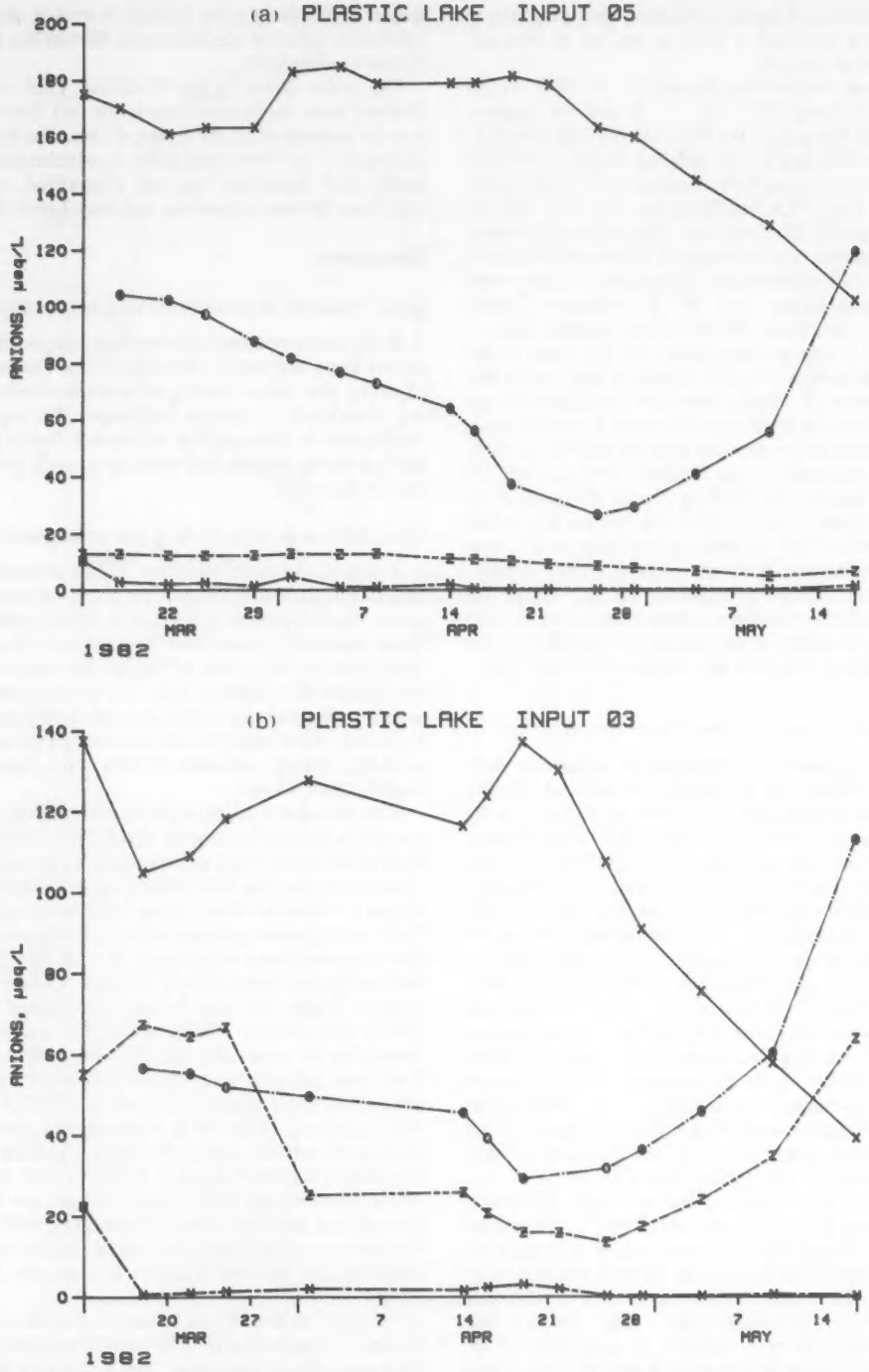
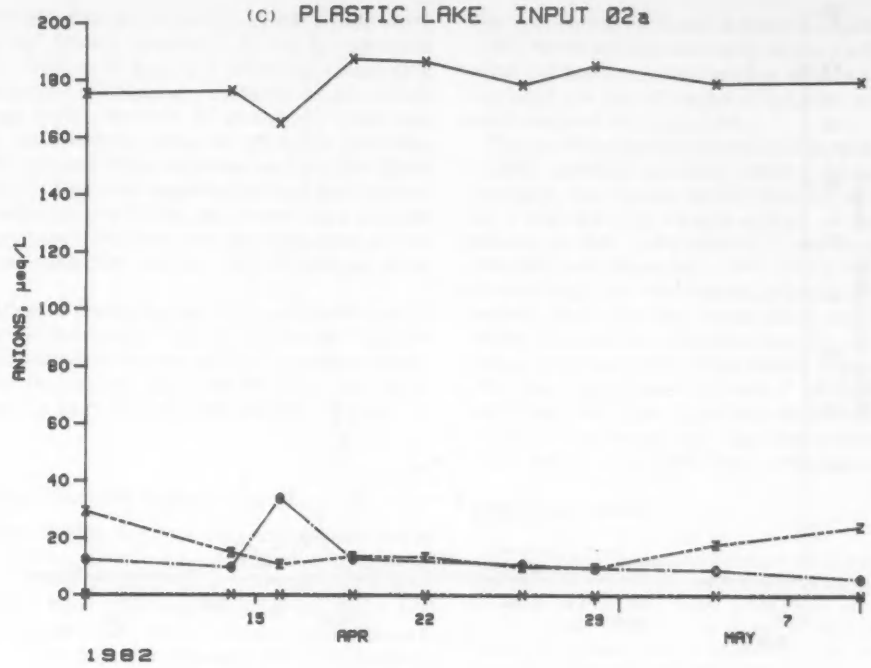


FIG. 3. Anion equivalents for four other streams (a, b, c, d) draining the Plastic Lake catchment; symbols and lines as in Fig. 2.



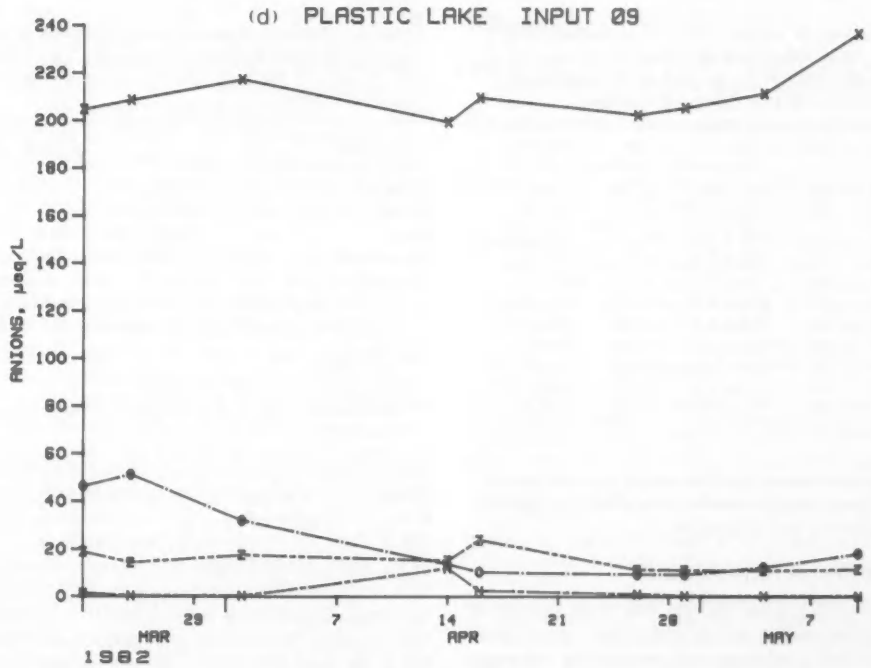


FIG. 3. (*Concluded*)

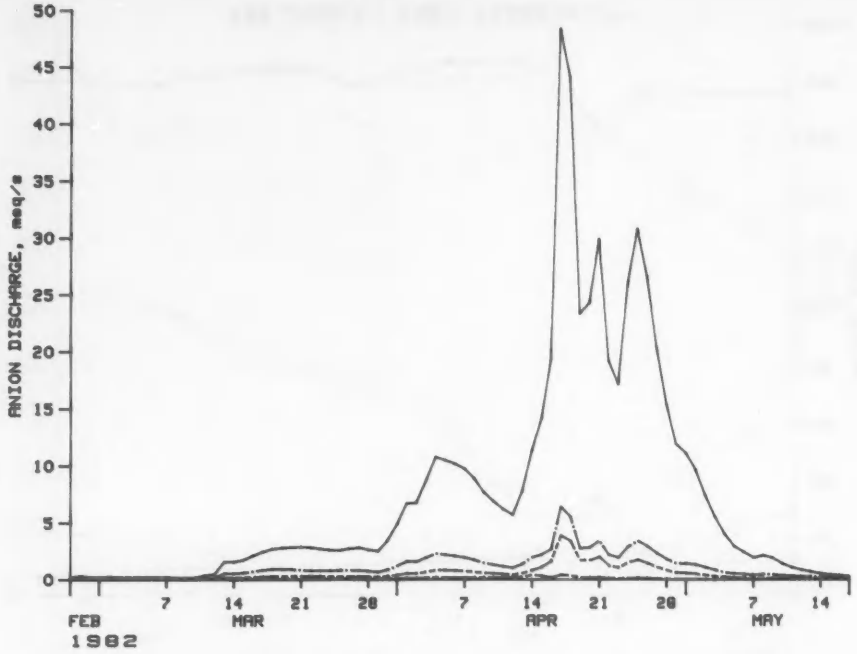


FIG. 4. Anion equivalents discharged into Plastic Lake during the spring, 1982. —, sulphate; —·—, organics; ——, nitrate; ——, other, primarily chloride and bicarbonate.

TABLE 1. Emissions of sulphur (10^6 t/yr) at Sudbury and average pH, H^+, and sulphate concentrations ($\bar{x} \pm$ SE; μeq/L) in Clearwater Lake over 11 yr, as well as the number of samples collected per year (n). NA = not available.

Year	SO_2	n	$[H^+]$	pH	$[SO_4^{2-}]$
1973	1.46	16	51.3±3.2	4.29	579±7
1974	1.47	23	57.5±2.4	4.24	533±9
1975	1.39	13	46.8±1.6	4.33	519±14
1976	1.41	20	58.9±2.5	4.23	542±7
1977	1.34	11	79.4±1.8	4.10	550±7
1978	0.68	12	39.8±1.8	4.40	498±4
1979	0.50	6	38.9±4.5	4.41	458±4
1980	0.94	12	33.1±2.4	4.48	457±5
1981	0.84	7	33.1±2.9	4.48	408±6
1982	0.39	6	33.1±1.6	4.48	396±3
1983	NA	6	25.1±2.2	4.60	379±5

TABLE 2. Number of years of data collection (n), atmospheric deposition of strong acid (D), flux ($meq \cdot m^{-2} \cdot yr^{-1}$) of basic cations ($M^+ = Ca^{2+} + Mg^{2+} + Na^+ + K^+$), and bicarbonate in three regions of Ontario.

Catchment	n	D	M^+ flux[a]	HCO_3^- flux
Rawson[b]	3	8–10	33	14
Dickie[c]	4	70–100	67	14
Harp[d]	4	70–100	111	32
Clearwater[e]	2.5	200	116	2

[a]Net flux = gross flux less input from atmospheric deposition.
[b]Schindler et al. 1976; D. W. Schindler and S. Bayley, pers. comm.
[c]This study.
[d]This study; Dillon et al. 1982.
[e]Jeffries et al. 1984; Ontario Ministry of the Environment 1982b.

catchments in central Ontario. In other areas, however, anthropogenic nitric acid may play a more important role during early spring runoff (Galloway and Dillon 1983).

Does Humic Acid Replace Sulphuric Acid?

Krug and Frink (1983) have implied that if the loading of anthropogenic mineral acids to currently acidified ecosystems is reduced, the acidified catchment will respond by releasing equivalent amounts of humic (not fulvic) acid. To replace the anthropogenic sulphate loaded into Plastic Lake during peak flow periods with equivalent amounts of humic acid would require, however, unreasonable quantities: 35–40 mg C/L (using the Oliver et al. model, pH = 4.5, sulphate = 180 μeq/L, B = 7.7 μeq/mg C of humid acid). Although organic acids are involved in cation transport between well-drained soil horizons in some pristine forested systems (Ugolini et al. 1977; Cronan et al. 1978; Dawson et al. 1981; McDowell 1982), these are thought to be fulvic acids, not humic, and they rarely reach groundwaters and streams draining the mineral soils (Johnson 1981; Dawson et al. 1981; McDowell 1982), apparently precipitating in the B horizon. Waterlogged swamp soils, as noted here also, are an exception. Although a definitive test of the Krug and Frink hypothesis cannot be made with the Plastic Lake data, the hypothesis does appear improbable. A more likely scenario following the reduction of sulphate deposition to the Plastic Lake catchment would be a reduced spring pH depression in clear streams and the emergence of a spring pH elevation in the colored streams because of the dilution of organic acids by spring snowmelt.

The Sudbury lake data can be used to provide a more direct test of Krug and Frink's hypothesis. If one accepts their arguments, the decrease in local SO_2 emissions should have resulted in an increase in organic acid content in the lakes rather than an increase in pH. However, no change in organic acid concentration was observed, while the pH of the two lakes studied, Clearwater and Swan, increased sutstantially. These results indicate that there is no counterbalancing effect between sulphate flux and organic acid flux; an increase in one does not necessitate a decrease in the other. For these lakes, then, and we suspect for many others, the Krug and Frink hypothesis can be rejected.

The implications of this finding are important: acidification of freshwaters, even those as severely affected as the Sudbury lakes, is at least partially reversible. It is not yet known whether these beneficial changes in water chemistry can result in the reversal of some or all of the biological damage done to these lakes.

Are Basic Cation Fluxes and Sulphate Levels Low?

It is well documented that sulphate concentrations in the freshwaters of central Ontario have increased as a result of atmospheric deposition. Softwater lakes in Precambrian regions of North America where the deposition of strong acid is low (typically $<15\ meq \cdot m^{-2} \cdot yr^{-1}$) have sulphate concentrations of 20–70 μeq/L (Armstrong and Schindler 1971; Duthie and Ostrofsky 1974; Watt et al. 1979; reviewed in Harvey et al. 1981). The few exceptions are those where sea salts contribute greatly to the total ionic strength of the lake, or where sedimentary deposits high in major ion content exist in the catchment. In contrast, lakes in Precambrian regions of central Ontario have sulphate levels of 160–220 μeq/L (Dillon et al. 1978), roughly 3–10 times "background" levels. We have shown that a decrease in organic anion flux is unlikely as a consequence of the increase in sulphate. Rather, the increased sulphate concentration must be balanced by an alkalinity decrease and/or increased basic cation levels resulting from the increased flux from their catchments.

However, Krug and Frink (1983) contend that the rates of basic cation export from catchments receiving substantial amounts of acidic deposition are "remarkably" low. We have shown here that of the three regions studied (where the soils are typically of low pH) the flux of basic cations was greatest in the two where the deposition rate of acid was highest ($>70\ meq \cdot m^{-2} \cdot yr^{-1}$). The basic cation flux was largest in the Sudbury area where the deposition rate of acid was the highest, even though the proton flux had become increasingly important. The basic cation flux in the area with the lowest deposition of strong acid (Rawson Lake) was greater than the input rate of strong acid ($33\ meq \cdot m^{-2} \cdot yr^{-1}$ versus $8–10\ meq \cdot m^{-2} \cdot yr^{-1}$), indicating that natural processes control basic cation flux at this location. In central Ontario (Harp and Dickie Lake catchments), the basic cation flux is clearly controlled by the strong acid input and mobile anion (SO_4^{2-}) output; the natural processes must make a relatively small contribution. We conclude therefore that in acidic soils, basic cation flux is increased as a result of the deposition of strong acids from the atmosphere. The fact that the basic cation flux was not directly proportional to the strong acid deposition rate at these locations cannot be considered an indication that the fluxes were unaffected by acidic deposition. Basic cation flux resulting from natural processes (weathering by H_2CO_3 or by organic acids) is less significant quantitatively than that caused by acidic deposition. Gorham and Gordon (1960) also noted that lakes near Sudbury with pH < 5.0 had higher calcium concentrations than those with pH > 6.0 and concluded that the catchments of the lakes with lower pH had been weathered at a greater rate.

The fact that sulphate concentration in freshwaters subjected to acidic deposition increases without a corresponding decrease in organic acid content necessitates that alkalinity decreases (or is superseded by mineral acidity), or basic cation levels increase, or that a combination of both occur. The former is consistent with Henriksen's (1979, 1980) concept of acidification as a large-scale acid titration, whereas the latter possibility suggests that assimilation of the strong acid by exchange for cations occurs in the catchment, resulting in increased cation fluxes (Henriksen 1982). The relative importance of the two processes, mineral acid titration of alkalinity and increased cation flux, determines how fast a freshwater system loses its acid neutralization capacity. That either process occurs appears to be contrary to Krug and Frink's arguments.

Acknowledgments

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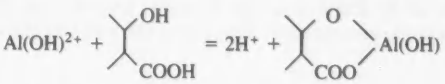
Appendix

Using the complete suite of chemical parameters that we have estimated (see Methods), a detailed inventory of inorganic cations and anions can be constructed and the concentration of organic acid anions (and the free protons released by them) estimated by difference (Cronan et al. 1978). However, it is not only the "free" organic acid anion that has released a proton into solution but also the organics that are currently complexed to metals; it is thus of interest to estimate the abundance of both these components. Flame atomic absorption measures both the organically bound and free di- and mono-valent metals (Ca,

Mg, Mn, Na, K). As these are most likely organically bound (if at all) as outer sphere or weak field (e.g. Bloom 1981), mono- or di-carboxylic complexes (i.e. one dissociated carboxyl group per charge per atom), the sum of these cation equivalents after subtracting the sum of the inorganic anion equivalents will provide an estimate of the carboxyl group equivalents of both the free organic anion as well as that bound to these metals. Iron and aluminum, however, may be bound as inner sphere type I complexes (Schnitzer and Khan 1972; Gamble and Schnitzer 1973; Gamble et al. 1980) of the salicylic acid type:



with two protons released but only one carboxyl group complexed. However, the evidence supporting this molecular model can be criticized (Bloom 1981); an alternative is the phthallic acid dicarboxylic complexation model (type II) where two protons are released as before but two carboxyl groups are complexed. Organically bound aluminum was separated by dialysis followed with an oxine extraction (LaZerte 1984). Total iron was measured by flame atomic absorption, and assuming that all this iron is organically bound, rather than being present as colloidal, peptized iron oxides (Perdue et al. 1976). Evidence will be provided later in the appendix as independent support for this assumption. Monomeric inorganic iron can be ignored at these pH's. The moles of organic iron and aluminum estimated in this way can then be doubled (two protons released per metal atom) and added to the simple charge difference already described to obtain an estimate of total protons released from dissolved organic acids. The major problem with this method is that sums and differences of many numbers are necessarily quite variable and rather expensive and time consuming to obtain. Nonetheless, this difference provides one estimate of the protons released into solution by organic acids.

A second method of estimating organic proton release is based on an empirical estimate of the abundance of dissociated and complexed carboxyl groups in solution (Oliver et al. 1983). They have estimated the total carboxylic acid content (B) of humic and fulvic acids (FA, mg C/L) over a wide range of natural waters as 10.5 ± 1 μeq/mg C; for fulvic acid alone, the value of B was 10.7 ± 1 μeq/mg C. The organic acids used in these determinations were purified and stripped of complexed metals. The authors also provided an empirical estimate of the dissociation constant (K_h) of these carboxylic acid groups as a function of pH:

$$(1)\quad K_h = 0.96 + 0.90\cdot\mathrm{pH} - 0.039\cdot\mathrm{pH}^2$$

and concluded that, in the absence of complexing metals,

$$(2)\quad \mathrm{A}^-(\mu\mathrm{eq/L}) = (K_h/(K_h + \mathrm{H}^+))\cdot C_t$$

where A^- represents the dissociated or free organic anions (and thus the amount of protons released) and $C_t(\mu\mathrm{eq/L}) = B\cdot\mathrm{FA}$. If some of the carboxyl groups are complexed by metals (A^c, μeq/L), they argue that

$$(3)\quad \mathrm{A}^- + \mathrm{A}^c(\mu\mathrm{eq/L}) = (K_h/(K_h + \mathrm{H}^+))\cdot C_t.$$

Assuming the type II dicarboxylic complexation model for iron and aluminum, the charge balance constructed earlier should be equivalent to the value of $A^- + A^c$, as each proton released in the complexation reaction is derived from carboxyl groups. This equivalence provides one test of the Oliver et al. model. Assuming the type I complexation model, however, the charge balance constructed earlier must be modified by adding only equimolar amounts of organic iron and aluminum to the balance, rather than double this, because only one proton is released by a carboxyl group. This will provide an alternative evaluation of the Oliver et al. model, but does ignore any potential release of protons from complexed phenolic sites.

Possibly, rather than equation 3, a model more appropriate for the tightly bound, type I complexes would be

$$(4)\quad \mathrm{A}^-(\mu\mathrm{eq/L}) = (K_h/(K_h + \mathrm{H}^+))\cdot(C_t - \mathrm{A}^c).$$

Unfortunately, this model cannot be tested here, as the charge balance method described earlier only provides estimates of $A^- + A^c$; it does not permit the separation of the two groups because we have no separate estimates of organically bound Ca, Mg, Mn, Na, and K. However, as will be shown later (Fig. A.1), equation 3 fits the data very well.

The final consideration with respect to the Oliver et al. model is that it requires an estimate of fulvic acid (FA) content. The DOC estimates described earlier must overestimate the FA fraction; thus, we assume a fixed amount of non-fulvic acid carbon in all waters analyzed:

$$(5)\quad \mathrm{DOC\ (mg\ C/L)} = D + \mathrm{FA}$$

where D is expected to be some small positive number (mg C/L). Equation 3 can now be re-written as

$$(6)\quad (\mathrm{A}^- + \mathrm{A}^c)\cdot(K_h + \mathrm{H}^+)/K_h = B\cdot(\mathrm{DOC} - D) = D' + B\cdot\mathrm{DOC}$$

where $D' = -D\cdot B$. This is the equation that will be used to verify the Oliver et al. model. Using standard linear regression techniques, estimates can be obtained for D' and B; the values of B can then be compared with those reported by Oliver et al. and the value of D' can be used to estimate D, the amount of non-fulvic acid carbon. However, D' might also be influenced by some constant, systematic error in the charge balance. As is appropriate for a statistical procedure of this type, the left-hand side of equation 6 contains the majority of the random error, as it is a function of many variables in the charge balance; the right-hand side, however, contains only one variable (DOC), which will be assumed to be relatively error free.

The Oliver et al. model (equation 3) was first verified in the regression format of equation (equation 6) and assuming only type I aluminum and iron complexation sites. Stream samples were collected from 10 different streams in the Muskoka–Haliburton region of central Ontario from March to May of 1982, including the streams in Plastic Lake catchment described earlier. The estimated value of B was 10.9 ± 0.6 (mean ± 95% C.I.; $R^2 = 0.84$, $n = 257$; Fig. A.1), which compares favorably with the value obtained by Oliver et al. of 10.7 ± 1.0 for fulvic acids alone and 10.5 ± 1.0 for all extractable organic acids. From the estimated intercept (−13.7 ± 3.7) it can be calculated that there is, on the average, 1.5 mg non-fulvic acid carbon/L in these waters. Two outliers (seen in the upper left-hand corner of Fig. A.2) were found to have levels of iron higher than could be complexed by the number of carboxyl groups present; as both samples came from unfiltered soil pit water, we assumed that they were contaminated with iron oxides. Most of the other samples had levels of iron and organic aluminum per milligrams carbon of DOC lower than 3 μmol/mg C (Fig. A.2), the expected number of type I sites for a soil fulvic acid (Gamble and Schnitzer 1973).

The type II complexation model does not provide as close a fit to the Oliver et al. data; the estimated value of B was 12.5 ± 0.7

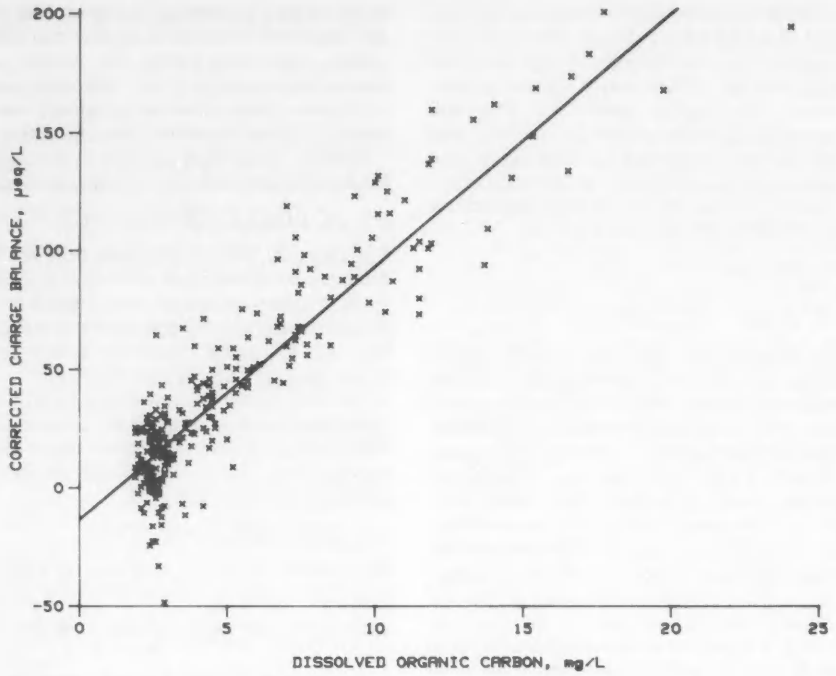


FIG. A.1. Corrected charge balance (left-hand side of equation 6 from the appendix) versus dissolved organic carbon (right-hand side of equation 6).

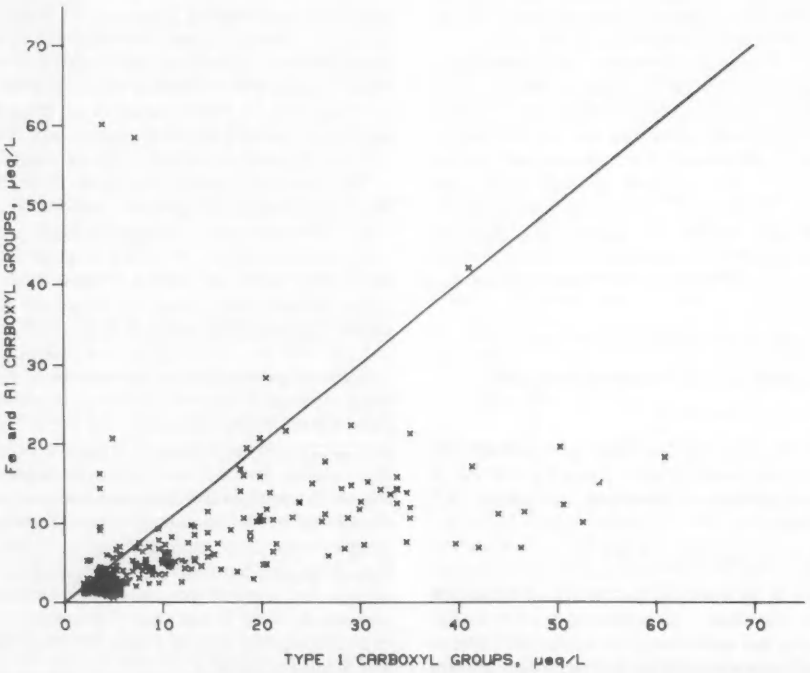


FIG. A.2. Estimated number of carboxyl groups complexed with iron and aluminum in salicylic acid type I groups versus the total number of type I carboxyl groups expected (3 µeq/mg C).

(mean ± 95% C.I.; $R^2 = 0.84$, $n = 257$), although it is still reasonably close. The intercept was not significantly different (-12.9 ± 4.8).

Taken together, these results suggest that the type I complexation model for iron and aluminum and the Oliver et al. empirical model for carboxylic acid functional group dissociation are appropriate for the natural waters considered here. Using the Oliver et al. model we can then estimate the number of protons contributed by both dissociated and complexed carboxylic acid functional groups. The model does not, however, predict the protons released by complexed phenolic acid functional groups in the type I sites. If the amount of organically bound aluminum and iron is known, this can be estimated separately; but the attractiveness of the Oliver et al. model is in its simplicity, and for some of our samples no estimates of organically bound aluminum or iron are available. We have thus chosen to ignore the possible contribution of complexed phenolic groups to the organic sources of acidity. We can estimate our average error by regressing the charge balance, which does include all protons released, against the Oliver et al. model, which does not. This analysis suggests that we could be underestimating the total protons released by organics by about 13% (±5%).

Electrolyte and Water Balance in Plasma and Urine of Rainbow Trout (*Salmo gairdneri*) during Chronic Exposure to Cadmium

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Giles, M. A. 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Can. J. Fish. Aquat. Sci. 41: 1678–1685.

Rainbow trout (*Salmo gairdneri*) were exposed to 3.6 and 6.4 μg Cd/L for periods up to 178 d. Transitory changes in plasma calcium and magnesium were observed in fish exposed to 3.6 μg Cd/L although the differences were not significant. Exposure to 6.4 μg Cd/L, however, resulted in significantly lowered plasma sodium, potassium, calcium, and chloride and elevated magnesium concentrations. Analyses of urine indicated that the rate of urine production, osmolality, and sodium, potassium, chloride, magnesium, calcium, and protein concentrations were unaffected by exposure to 3.6 μg Cd/L although slight changes were observed in the first week of exposure. Urine production rate and urinary concentrations of potassium and chloride were unaffected in trout exposed to 6.4 μg Cd/L but sodium, protein, and osmolality were elevated and calcium and magnesium concentrations reduced in these fish. The results demonstrate that the majority of the cadmium-induced electrolyte imbalances do not result from impairment of renal function.

Des truites arc-en-ciel (*Salmo gairdneri*) ont été exposées à 3,6 et 6,4 μg Cd/L pendant des périodes pouvant atteindre 178 d. A 3,6 μg Cd/L on a observé des changements transitoires de la teneur en calcium et en magnésium du plasma, mais les différences n'étaient pas significatives. Toutefois, à 6,4 μg Cd/L la baisse de sodium, de potassium, de calcium et de chlorure dans le plasma était significative, tout comme l'augmentation de la concentration en magnésium. L'analyse des urines à montré que la production d'urine, l'osmolalité, et les concentrations de sodium, potassium, chlorure, magnésium, calcium et protéines n'étaient pas affectées par l'exposition à 3,6 μg Cd/L, en dépit de légers changements la première semaine. A 6,4 μg Cd/L, la production d'urine et la concentration de potassium et de chlorure dans l'urine n'étaient pas modifiées, mais le sodium, les protéines et l'osmolalité augmentaient alors que le calcium et le magnésium diminuaient. Ces résultats démontrent que la majorité des déséquilibres électrolytiques induits par le cadmium ne proviennent pas de lésions de la fonction rénale.

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Dissolved cadmium is extremely toxic to salmonids in freshwater with median lethal concentrations (LC50) for adult fish ranging from 3.7 μg/L for coho salmon, *Oncorhynchus kisutch* (Chapman and Stevens 1978), to 10–15 μg/L for rainbow trout, *Salmo gairdneri* (Ball 1967; Majewski and Giles 1981). Sublethal effects on salmonids have been observed at cadmium concentrations of 3.4 μg/L (Benoit et al. 1976) and 6.4 μg/L (Majewski and Giles 1981), which represent approximately 20 and 40%, respectively, of the incipient lethal concentrations (ILL) to rainbow trout (Majewski and Giles 1981). Levels of dissolved cadmium in this range have been recorded in surface waters near base metal smelting operations (McFarlane and Franzin 1978), electroplating industries (Murphy et al. 1978), and in isolated watersheds contaminated through atmospheric transport of pollutants from a mixture of industrial activities (Henriksen and Wright 1978).

In light of the sensitivity of salmonids to cadmium and the level of contamination associated with metal production, it is surprising that relatively little is known regarding the mechanisms of its toxic action. Cardiovascular–respiratory impairment (Bishop and McIntosh 1981; Majewski and Giles 1981), blood electrolyte changes (McCarty and Houston 1976; Christensen et al. 1977), enzymatic and hormonal changes (Christensen 1975), increased locomotor activity (Benoit et al. 1976; Ellgaard et al. 1978), and retardation of growth (Benoit et al. 1976) have been reported for fish exposed to sublethal concentrations of cadmium. Whereas the diverse effects or symptoms of cadmium intoxication have been described the physiological mechanisms whereby these effects arise are largely unknown.

Blood electrolyte regulation in freshwater fish is a result of several interacting processes: absorption of electrolytes from water by active transport mechanisms, predominantly at the gill; control of water permeability of the body surface, especially the gill; and selective reabsorption of electrolytes from urine as osmotically gained water is excreted. A stress that impairs one or more of these elements will result in a change in the concentrations of various plasma electrolytes or a compensatory change in one or both of the other processes. Degenerative, cadmium-induced renal changes have been reported for mam-

mals (Nishizumi 1972; Singhal et al. 1974; Kotsonis and Klaassen 1978). Gardner and Yevich (1970) reported pathological changes in the proximal tubules but not the glomeruli of killifish, *Fundulus heterolitus*, exposed to high concentrations of cadmium in seawater. My study was undertaken, therefore, to examine the effects of long-term sublethal exposure to cadmium on plasma electrolyte and to identify the role of the kidney in these effects.

Methods

Rainbow trout with a mean (±SD) weight of 375 ± 92g were obtained from the Rockwood Experimental Fish Hatchery, Rockwood, Manitoba. Sixteen fish were placed in each of six 200-L fiberglass tanks and acclimated to dechlorinated Winnipeg City water (Ca = 23.6 mg/L; Mg = 5.4 mg/L; Na = 1.8 mg/L; total inorganic carbon = 17.2 mg/L; pH = 7.8; hardness = 82 mg/L as $CaCO_3$) at a 12 h light : 12 h dark photoperiod for 4 mo and subsequently exposed to total dissolved cadmium concentrations of 3.6 ± 0.4 and 6.4 ± 0.5 μg/L (mean ± SE) for periods up to 178 d. The cadmium concentration in control tanks was less than 0.1 μg/L. Water temperature was maintained at 10°C except for the last 3 wk of cadmium exposure when the temperature rose to 15°C at the rate of 0.25°/d. Fish were fed commercial pellets once daily for 5 d/wk at a rate of 2% body weight/d.

Urine was collected from five fish from each test condition at intervals over the 178-d experimental period. Each fish was anesthetized with MS-222 (0.01% solution) and a water-filled, PE-90 polyethylene catheter inserted into the urinary bladder. A sleeve of silicone tubing, applied over the catheter and secured tightly against the urinary papilla, was sufficient to prevent leakage of urine at an externally applied pressure of 15 cmH_2O. The catheter was sutured firmly at the anterior and posterior boundaries of the anal fin. The fish were then placed into the restraining chambers described by Majewski et al. (1979) and returned to their respective exposure tanks. The urinary cannula was placed into a collection vessel such that the cannula opened 3 cm below the water level of the aquarium. The fish were allowed to recover from surgical trauma for 3 d after which 24-h composite samples of urine were collected for an additional 3 d. Total urine flow and chemical composition were measured for each of the 3 d. The urine was stored for up to 6 d at 2°C prior to analysis.

Following the final urine sampling the fish were anesthetized with MS-222 (0.02% solution). Blood was obtained from the dorsal aorta in the roof of the mouth with a heparinized syringe (approximately 50 units ammonium heparin/L). The blood was centrifuged at 12 800 × *g* for 2 min and the plasma collected by aspiration. The plasma was stored at 2°C and chemical analyses performed within 5 d. The fish were killed after bleeding.

Chemical analyses were done on urine and plasma. Sodium and potassium were measured in triplicate 20-μL aliquots by flame photometry (Beckman KliNa-Flame). Chloride was determined coulometrically with a Radiometer CMT-10 chloride titrator. Calcium and magnesium were measured by atomic absorption at 422.7 and 285.2 nm, respectively, with a Unicam SP90 spectrophotometer. Phosphate interference with calcium analyses was suppressed by the addition of lanthanum (final concentration of 1% La by weight). Plasma and urine osmolalities were measured in duplicate on 250-μL samples by freezing point depression (Advanced Instruments Inc., model 3R osmometer). Plasma protein was determined in duplicate by the Biuret method (Henry 1964) whereas urine protein was determined by the phenol method (Henry 1964).

The results from the plasma and urine analyses were subjected to a two-way analysis of variance (ANOVA) for treatment and time effects. Since Bartletts test of homogeneity of variance (Snedecor and Cochrane 1967) was significant for most of the urinary data, all urine results were subjected to a logarithmic transformation prior to ANOVA using means of the individual daily urine concentrations (to calculate electrolyte concentration) or the mean of the product of the daily electrolyte concentration and the respective flow rate (to calculate electrolyte output) over the 3-d collection period. All proportional data were subjected to angular transformation prior to analyses of variance. The data are expressed as the arithmetic mean ± 1 SE.

Results

Plasma

None of the plasma constituents were significantly different from control values in fish exposed to 3.6 μg Cd/L over the experimental period, although $[Na]_p$, $[Ca]_p$, and $[Cl]_p$ were reduced at day 3 (Table 1). The fraction of the plasma osmolality accounted for by the five measured electrolytes (EOF), assuming complete ionic dissociation, was slightly different from controls in the low-cadmium group. Since the largest difference in EOF between controls and the 3.6 μg Cd/L group occurred during the 178-d sampling period when temperature control was impaired, this effect may not be a simple response to cadmium.

Exposure to 6.4 μg Cd/L resulted in reduced plasma sodium, potassium, calcium, and chloride and increased plasma magnesium. These changes were relatively small and did not appear to vary with time of exposure (Table 1). Neither protein concentration nor plasma osmolality was significantly different from control values in the fish exposed to 6.4 μg Cd/L. In view of the average decrease in blood electrolytes of 9.8 mmol/L after correction for the increase in $[Mg]_p$, the lack of a significant reduction in plasma osmolality was unexpected. This discepancy is reflected in the highly significant effect upon EOF of trout exposed to 6.4 μg Cd/L. Clearly, some osmotic constituent of plasma must have increased by approximately 7.7 mmol/L, since osmolality only declined by an average of 2.1 mosmol/kg in cadmium-exposed fish. In a preliminary study, bicarbonate ion increased by 1.5 mmol/L in fish exposed to 4 μg Cd/L for 126 d (M. A. Giles, unpubl. obs.) and could not account, therefore, for a major proportion of the unidentified osmotic constituents in fish exposed to 6.4 μg Cd/L.

Significant time effects were observed in all plasma factors except $[K]_p$ and the $[Cl]_p$ of the 6.4 μg Cd/L group. These effects may reflect seasonal or reproductive changes in electrolyte balance. In general the highest concentrations of the electrolytes occurred in the 52-d samples (mid-March). Although the time-related variation often exceeded the treatment effects there was no apparent relationship between the exposure time and the degree of the treatment effects.

Urine

Cadmium exposure at 3.6 μg/L failed to elicit a significant change in the urinary concentrations of any of the five

TABLE 1. Effects of chronic cadmium exposure upon plasma electrolytes, protein, and osm
wise in parentheses.

	[Cd] (μg/L)	−9 to −11	3	10	
Na^+ (mmol/L)	0	154.0±1.0(6)	155.2±1.1	149.8±2.3	14
	3.6	—	151.9±1.8	149.2±1.3	15
	6.4	—	154.0±1.7	145.7±2.9(4)	14
K^+ (mmol/L)	0	2.79±0.24(6)	2.72±0.15	3.02±0.3	2
	3.6	—	2.93±0.13	2.50±0.07	2
	6.4	—	2.56±0.10	2.68±1.5(4)	2
Ca^{2+} (mmol/L)	0	—	2.12±0.03(4)	2.16±0.03	2
	3.6	—	1.67±0.08	2.19±0.08	2
	6.4	—	1.45±0.04	1.82±0.12	1
Mg^{2+} (mmol/L)	0	—	0.89±0.01(4)	0.69±0.02	0
	3.6	—	0.93±0.03	0.76±0.05	0
	6.4	—	1.03±0.06	0.91±0.04	0
Cl^- (mmol/L)	0	130.7±2.4(6)	135.6±1.2	128.8±2.0	13
	3.6		130.0±2.5	134.0±1.5	12
	6.4		127.8±1.3	129.5±2.2(4)	13
Osmolality (mosmol/kg)	0	308.5±0.9(6)	306.0±1.1	301.0±3.7	30
	3.6		304.1±2.3	302.6±1.8	30
	6.4		305.1±2.6	301.0±3.0(4)	30
Protein (g/dL)	0	2.99±0.50(6)	3.43±0.58	3.05±0.31	3
	3.6		3.15±0.29	3.42±0.17	3
	6.4		3.42±0.23	3.24±0.22	3
Electrolyte osmotic fraction (%)[d]	0	—	97.6±0.6(4)	94.5±0.7	9
	3.6	—	94.5±0.8	95.4±0.3	9
	6.4	—	94.0±0.5	94.0±0.7(4)	9

[a]Levels of significance in ANOVA in parentheses: (0) not significant; (1) $0.1 \geq P > 0.01$; (2) $0.01 \geq$
[b]Degrees of freedom treatment (1, 46–51).
[c]Degrees of freedom time (5, 46–51).
[d]Electrolyte osmotic fraction = sum of plasma electrolyte concentrations (mmol/L) 100/plasma osmol

and osmolality in rainbow trout. Results are mean ± 1 SE for $n = 5$ except where indicated other-

	Exposure time (d)				*F*-ratio[a]		
	24	52	129	178	Treatment[b]	Time[c]	Bartlett's χ^2 value[a]
3	149.9±2.3(4)	158.3±1.1	155.7±2.5	147.8±2.5(4)			
3	150.8±0.5	155.6±1.7	158.0±0.9	148.0±2.2(4)	0.45(0)	9.94(3)	14.06(0)
9(4)	147.7±1.4	152.6±1.1	142.5±2.5(4)	148.0±1.4(4)	12.78(3)	5.32(3)	9.37(0)
3	2.64±0.15(4)	2.70±0.07	3.17±0.9	2.99±0.26			
07	2.59±0.11	2.69±0.11	2.75±0.14	2.75±0.13	5.13(1)	1.60(0)	17.25(0)
5(4)	2.51±0.08	2.55±0.14	2.51±0.16(4)	2.48±0.04	18.46(3)	1.76(0)	16.62(0)
03	2.24±0.05(4)	2.72±0.23	2.37±0.05	2.29±0.06			
08	2.15±0.05	2.87±0.20	2.23±0.03	2.27±0.04	2.39(0, 1)	15.28(3)	39.08(3)
12	1.98±0.11	2.42±0.23	2.03±0.12(4)	2.22±0.15	23.32(3)	9.97(3)	31.06(3)
02	0.89±0.03(4)	0.92±0.05	0.80±0.01	0.82±0.02			
05	0.86±0.02	0.89±0.02	0.78±0.02	0.89±0.05	1.42(0)	10.37(3)	24.36(2)
04	0.98±0.06	1.05±0.05	0.82±0.03(4)	1.13±0.10	31.90(3)	6.09(3)	29.32(3)
0	130.0±4.1(4)	134.8±2.5	132.6±2.5	131.3±2.0(4)			
5	129.6±2.8	132.2±1.8	138.6±1.7	124.4±2.2	0.33(0)	2.69(1)	6.48(0)
2(4)	130.6±1.9	128.6±2.5	118.8±3.4(4)	128.3±1.7(4)	10.90(2)	1.22(0)	7.27(0)
7	301.8±3.5(4)	311.4±1.6	316.9±4.2	303.6±2.8(4)			
8	305.4±1.4	309.7±2.6	318.9±1.8	308.2±3.7(4)	0.47(0)	10.52(3)	12.57(0)
0(4)	303.8±2.1	305.2±1.8	300.0±4.8(4)	314.4±2.0(4)	1.23(0)	1.99(1)	12.00(0)
31	3.88±0.20(4)	4.01±0.38	4.41±0.20	3.40±0.40			
17	3.96±0.15	4.07±0.41	3.81±0.25	4.10±0.28	0.11(0)	2.68(1)	12.87(0)
22	3.96±0.37	3.92±0.28	4.20±0.24(4)	5.42±0.37	2.36(0)	3.55(2)	9.70(0)
7	94.6±1.2(4)	96.2±0.6	93.0±0.7	94.0±0.9(4)			
3	93.7±0.9	95.0±0.7	94.8±0.9	90.8±0.9(4)	4.10(1)	3.10(1)	4.38(0)
7(4)	93.4±0.4	94.1±0.9	88.8±0.5(4)	90.2±0.6(3)	31.88(3)	6.59(3)	7.63(0)

) $0.01 \geq P > 0.001$; (3) $P \leq 0.001$.

na osmolality.

TABLE 2. Effects of chronic cadmium exposure upon the concentrations of electrolytes and protein, osmolality, and production rates of urine in rainbow trout. Results are mean ± 1 SE for $n = 4–5$. Analyses of variance were calculated after log transformation of the average concentrations and urine volumes recorded in individual fish over the 3-d interval of each sampling period.

	[Cd] (μg/L)	Exposure time (d) 1–3	8–10	21–23	49–51	126–128	175–177	*F*-ratio[a] Treatment	Time	Bartlett's χ^2 value[a]
Na^+ (mmol/L)	0	11.8±3.5	9.5±0.5	13.5±0.5	9.0±0.8	10.0±1.7	7.3±1.4			
	3.6	17.3±1.6	11.7±1.3	13.0±1.9	14.0±2.2	9.0±2.0	6.8±2.0	1.44(0)	3.98(2)	17.8(0)
	6.4	20.0±3.7	21.5±5.2	13.8±2.3	15.8±2.6	11.4±2.1	7.2±0.6	9.61(2)	3.77(2)	16.9(0)
K^+ (mmol/L)	0	1.49±0.15	1.40±0.09	1.21±0.10	0.96±0.09	1.41±0.08	1.58±0.15			
	3.6	1.54±0.14	1.32±0.06	1.35±0.16	1.21±0.07	1.19±0.06	1.48±0.20	0.09(0)	3.84(2)	8.2(0)
	6.4	1.39±0.21	1.56±0.21	1.04±0.07	1.13±0.12	0.89±0.18	1.27±0.19	2.64(0)	2.51(1)	10.6(0)
Cl^- (mmol/L)	0	10.4±2.2	7.3±1.1	10.1±1.1	6.1±1.4	7.3±1.3	6.4±1.3			
	3.6	13.5±1.1	7.2±1.13	9.3±1.8	10.2±2.2	6.1±1.4	5.7±1.7	0.16(0)	3.47(2)	7.5(0)
	6.4	15.5±2.6	14.6±4.1	9.2±1.7	7.2±1.5	5.6±0.8	5.8±1.1	0.91(0)	4.19(2)	4.6(0)
Mg^{2+} (mmol/L)	0	1.42±0.65	0.71±0.12	0.92±0.11	0.87±0.18	0.53±0.07	0.44±0.06			
	3.6	2.14±0.68	0.56±0.17	0.85±0.11	0.79±0.15	0.59±0.08	0.69±0.05	0.22(0)	4.67(2)	21.1(1)
	6.4	0.97±0.20	0.76±0.12	0.54±0.12	0.36±0.06	0.65±0.16	0.61±0.14	2.97(1)	2.53(1)	7.3(0)
Ca^{2+} (mmol/L)	0	1.24±0.17	1.09±0.14	1.21±0.08	1.52±0.29	0.81±0.09	1.07±0.14			
	3.6	1.22±0.18	0.98±0.15	1.19±0.12	1.37±0.30	1.16±0.13	1.33±0.08	0.31(0)	1.72(0)	7.7(0)
	6.4	1.04±0.17	1.08±0.14	0.79±0.15	0.69±0.16	1.11±0.22	1.03±0.17	4.63(1)	0.42(0)	7.6(0)
Osmolality (mosmol/kg)	0	38.3±7.5	30.1±1.4	37.3±2.9	30.0±1.7	33.7±3.1	30.2±1.8			
	3.6	52.1±5.4	32.8±3.1	37.3±3.4	37.7±4.4	31.6±4.0	29.4±2.7	1.74(0)	3.02(1)	12.6(0)
	6.4	49.9±7.7	54.2±10.2	35.8±4.1	38.1±4.7	38.2±2.7	27.3±1.4	5.12(1)	2.29(1)	17.0(0)
Protein (mg/dL)	0	25.1±8.1	16.0±1.9	24.9±6.5	16.8±1.4	15.0±1.5	14.0±2.0			
	3.6	38.7±8.4	15.3±1.3	17.1±1.1	24.9±5.9	19.0±4.4	13.8±1.1	0.99(0)	3.16(1)	23.6(2)
	6.4	54.0±16.1	53.3±23.6	14.4±2.6	23.3±8.0	24.2±4.1	14.3±1.1	3.69(1)	2.34(1)	23.0(1)
Urine production ($mL \cdot d^{-1} \cdot kg^{-1}$)[b]	0	58.9±6.8	78.8±9.1	61.9±7.2	53.1±12.9	70.9±7.1	75.6±5.4			
	3.6	69.5±5.6	77.6±12.5	59.7±6.6	55.6±7.0	71.0±3.9	78.6±12.2	0.51(0)	2.57(1)	19.3(0)
	6.4	60.7±14.4	70.0±6.7	57.4±7.4	60.5±5.7	71.8±6.8	71.1±13.0	0.00(0)	1.52(0)	21.3(1)
Electrolyte osmotic fraction (%)	0	66.7±3.8	66.2±2.6	72.8±3.1	61.4±5.3	58.6±4.3	54.9±7.9			
	3.6	69.6±3.8	65.6±2.5	67.9±3.8	71.1±4.5	55.3±4.7	52.7±6.1	0.03(0)	4.06(2)	7.0(0)
	6.4	77.5±2.3	70.5±3.8	70.0±4.5	65.1±2.3	50.8±3.8	58.5±5.5	0.79(0)	5.20(3)	8.4(0)

[a]Levels of significance in ANOVA in parentheses: (0) not significant; (1) $0.1 \geq P > 0.01$; (2) $0.01 \geq P > 0.001$; (3) $P \leq 0.001$.
[b]Weights adjusted to 1 kg by $1000/W^{0.8474}$ where W = weight (g).

electrolytes or protein (Table 2). Large but transient increases were observed within the first 10 d of exposure to both cadmium concentrations, but only in the 6.4 μg Cd/L exposure were these maintained at a level to produce statistical significance. Of the monovalent electrolytes only $[Na]_u$ was elevated significantly at the high concentration of cadmium, and this increase was reflected in a significant elevation in osmolality.

Both the calcium, $[Ca]_u$, and magnesium, $[Mg]_u$, concentrations of the urine of fish exposed to 6.4 μg Cd/L were significantly different from controls. During the first 50 d of cadmium exposure, $[Ca]_u$ and $[Mg]_u$ averaged 73 and 69%, respectively, of control values. After longer periods of exposure, however, urinary concentrations of these divalent cations equalled or exceeded control values. Urinary protein concentrations were generally elevated in fish exposed to 6.4 μg Cd/L (Table 2). This effect was greatest during the first two sampling periods and was of borderline statistical significance. Since the variances were also nonhomogeneous, the significance of the response is questionable.

The EOF of urine averaged 64.2% over all treatments and sampling times and was not significantly different in control and cadmium-exposed fish (Table 2). The low EOF of urine is probably a reflection of the higher proportion of metabolic waste products present in urine relative to plasma. Inorganic phosphate concentrations of the urine of trout exposed to 6.4 μg Cd/L for 30 d and control fish were 4.48 ± 0.18 and 4.58 ± 0.26 mmol/L (mean ± 1 SE), respectively (M. A. Giles, unpubl. obs.) and could account for more than 10% of the EOF of urine.

The rate of urine excretion exhibited a strong mean–variance correlation in trout ranging in weight from 250 to 800 g. An analysis of covariance of log urine production versus log weight (Fig. 1) showed that the differences among slopes and adjusted group means were not statistically significant ($P < 0.01$), and the data were pooled to produce a single equation such that

$$(1) \quad U = 0.1885 W^{0.8474}$$

where U = urine production (millilitres per day) and W = weight of fish (grams). The correlation coefficient for this relationship was 0.476 for $n = 81$, which is highly significant ($P > 0.999$).

Although the rate of urine production, corrected to a common weight, was not influenced by cadmium treatment (Table 2), the results are somewhat confounded by the nonhomogeneity of variance between fish exposed to 6.4 μg Cd/L and controls. The

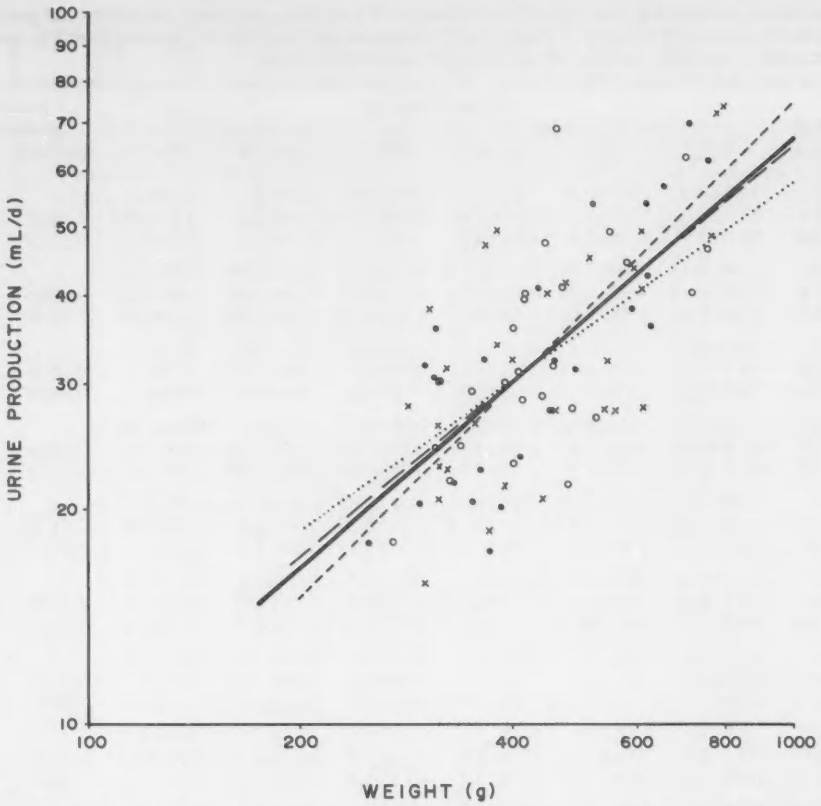

FIG. 1. Relationship between the rate of urine production and size in adult rainbow trout during the 178-d period. —, least squares fit for pooled observations from all treatment groups ($r^2 = 0.476$ for $n = 81$); ●---●, control, ×––×, 3.6 μg Cd/L; ○···○, 6.4 μg Cd/L.

time effect observed in the low-cadmium group was of borderline statistical significance and was not observed in the comparison of 6.4 μg Cd/L exposures and controls.

The total urinary excretion of electrolytes is a product of urinary electrolyte concentration and urine production rate. Since variation in total electrolyte excretion could be masked or accentuated by simple multiplication of mean rates of urine production and electrolyte concentration, the urinary electrolyte losses calculated from appropriate measurement on individual fish are presented in Table 3. I used equation 1 to adjust urine production to a common weight of 1 kg.

All treatment effects evident in the concentrations of urine electrolytes (Table 2), with the exception of sodium in the high-cadmium group, disappeared when daily urinary losses were calculated. This is, in part, a reflection of the higher variances associated with replication in the latter calculations. For example, the coefficients of variation associated with Na^+, K^+, Ca^{2+}, and protein in control urine were 28, 116, 23, and 22% higher, respectively, in urinary output calculations than in urinary concentration calculations. Possibly, total electrolyte loss was controlled to some degree by small, nonsignificant variations in the rates of urine production. Clearly, however, that sodium loss was significantly elevated in trout exposed to 6.4 μg Cd/L during the initial 50 d of exposure although these fish did return to sodium excretion rates that approximated control values in the 126–128 and 175–177 d sampling periods.

Discussion

Exposure to 6.4 μg Cd/L induced a slight, but significant, shift in the plasma electrolyte balance of rainbow trout. Reduced plasma concentrations of Na^+, K^+, Ca^{2+}, and Cl^- and elevated concentrations of Mg^{2+} were observed although total plasma osmolality was unchanged. These results support the observations of McCarty and Houston (1976) who reported reduced plasma levels of sodium and chloride in goldfish (*Carassius auratus*) exposed to 44.5 and 380 μg Cd/L for 25 and 50 d. Roch and Maly (1979) also reported severe hypocalcemia and elevated magnesium in the plasma of rainbow trout exposed to acultely lethal levels of cadmium. Effects such as these can result from impairment of net electrolyte influx at the gill, dilution of plasma by increased rates of water influx, redistribution of electrolytes between intracellular (ICC) or extracullular (ECC) compartments, impairments of renal function, or combinations of these processes. Assuming an ECC of 19% of body weight (Eddy and Bath 1979) which is chemically homogeneous with respect to the concentrations of major electrolytes and protein, my results can be employed to

TABLE 3. Excretion rates of electrolytes and protein in urine of rainbow trout c
were calculated after log transformation of the weighted excretion rates measure

	[Cd] (μg/L)	Exp 1–3	8–10	21–2
Na^+	0	608±124	763±114	842±1
($\mu mol \cdot d^{-1} \cdot kg^{-1}$)[a]	3.6	1190±119	849±60	786±1
	6.4	1093±261	1443±283	780±1
K^+	0	89.1±16.8	111.9±18.7	75.2±1
($\mu mol \cdot d^{-1} \cdot kg^{-1}$)[a]	3.6	106.5±11.3	102.3±16.3	83.2±1
	6.4	79.7±23.1	112.8±23.4	61.5±1
Cl^-	0	559±71	580±120	631±1
($\mu mol \cdot d^{-1} \cdot kg^{-1}$)[a]	3.6	927±81	502±39	574±1
	6.4	872±227	964±213	535±1
Mg^{2+}		77.5±31.3	57.4±14.6	56.0±5
($\mu mol \cdot d^{-1} \cdot kg^{-1}$)[a]	3.6	138.5±38.9	39.4±7.4	48.0±3
	6.4	58.4±20.1	55.4±14.1	30.5±7
Ca^{2+}		73.5±13.5	86.9±18.3	73.6±5
($\mu mol \cdot d^{-1} \cdot kg^{-1}$)[a]	3.6	84.4±16.0	72.2±10.2	69.8±7
	6.4	58.6±14.8	77.2±16.6	44.0±8
Protein	0	13.2±3.2	12.6±2.5	15.3±3
($mg \cdot d^{-1} \cdot kg^{-1}$)	3.6	26.2±5.1	11.4±1.3	10.4±1
	6.4	27.6±7.6	35.7±15.4	8.0±1

[a]Weights adjusted to 1 kg by $1000/W^{0.0474}$ where W = weight (g).
[b]Levels of significance in ANOVA in parentheses: 0, not significant; (1) 0.1 >

w trout chronically exposed to cadmium. Results are mean ± 1 SE for $n = 4–5$. Analyses of variance measured over the 3-d interval of each sampling period.

Exposure time (d)				F-ratio[b]		Bartlett's χ^2 value[b]
21–23	49–51	126–128	175–177	Treatment	Time	
842±116	486±116	693±103	573±132			
786±150	728±62	617±111	454±49	2.54(0)	2.35(1)	17.1(0)
780±127	1002±252	779±70	540±123	5.74(1)	2.04(1)	8.5(0)
75.2±11.3	50.1±14.0	102.0±14.0	119.4±14.0			
83.2±16.5	66.2±7.1	83.9±5.0	124.5±34.0	0.52(0)	4.58(2)	15.3(0)
61.5±10.8	66.8±6.1	63.6±15.1	99.8±27.2	0.92(0)	2.50(1)	13.6(0)
631±104	318±93	494±71	499±125			
574±135	508±67	416±84	387±49	0.70(0)	2.71(1)	19.0(0)
535±109	469±143	390±55	424±126	0.56(0)	2.92(1)	11.1(0)
56.0±5.8	45.3±10.8	35.4±1.4	34.3±6.1			
48.0±3.8	40.9±4.9	40.6±3.9	53.3±8.7	0.75(0)	3.03(1)	26.7(2)
30.5±7.0	22.1±4.7	49.5±15.7	45.3±14.4	2.08(0)	1.81(0)	17.3(0)
73.6±5.7	83.5±20.2	55.1±3.1	82.9±15.3			
69.8±7.6	71.2±11.6	81.0±8.5	105.8±20.1	0.84(0)	0.61(0)	22.4(1)
44.0±8.7	41.1±9.6	84.0±22.8	76.7±22.0	2.64(0)	0.91(0)	18.4(0)
15.3±3.8	9.2±2.4	10.6±1.3	10.7±1.9			
10.4±1.7	14.0±4.1	13.3±2.9	10.4±1.1	2.49(0)	2.27(1)	23.2(1)
8.0±1.3	15.9±7.2	17.2±3.0	10.6±2.4	.77(0)	2.02(1)	16.9(0)

(1) $0.1 \geq P > 0.01$; (2) $0.01 \geq P > 0.001$; (3) $P \leq 0.001$.

calculate the proportions of each component lost daily from the ECC through urinary excretion. Thus, for control fish, daily urinary excretion of sodium, chloride, protein, calcium, and magnesium comprised (mean ± 1 SD) 2.3 ± 0.5, 2.1 ± 0.4, 0.2 ± 0.05, 17.1 ± 2.7, and 34.5 ± 9.9%, respectively, of the amounts present in the extracellular fluid. These estimates for sodium and chloride are comparable with those calculated from estimates of electrolyte reabsorption from the gomerular filtrate in post-smolt rainbow trout (Holmes and Stainer 1966). To maintain homeostasis in the plasma these losses must be balanced by uptake mechanisms at the gill or through the diet. Since plasma calcium represents less than 3–6% of the total body calcium (Flemming 1974), this electrolyte may be maintained temporarily at normal levels by mobilization from other compartments such as bone or scales. The ECC and ICC of most freshwater teleosts, however, have approximately equal amounts of sodium and chloride (Holmes and Donaldson 1969) so there is little potential for offsetting losses of these electrolytes from the ECC by mobilization from the ICC.

Urinary sodium loss in trout exposed to 6.4 μg Cd/L exceeded that of control fish by an average of 525 μmol/d for the first 10 d of exposure. With an ECC equal to 19% of the body weight and a mean extracellular sodium concentration of 150 mmol/L in cadmium-exposed trout the total extracellular sodium content would total 28.5 mmol in a 1-kg fish. Clearly, without some compensatory mechanism in these fish, plasma sodium would be reduced by 18% within 10 d as opposed to the 3% decline actually observed. Using a net influx of sodium at the gill of approximately 130 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (McDonald and Wood 1981), a 17% increase in the net rate of sodium influx in cadmium-exposed fish would compensate for the increased urinary sodium loss. Watson and Beamish (1980) reported elevated levels in the ATPase systems responsible for the branchial influx of calcium, magnesium, and sodium in rainbow trout exposed to sublethal levels of zinc whereas Kench and Gubb (1970) reported a stimulatory effect of cadmium on erythrocytic ATPase of newly hatched chicks. A similar stimulatory effect of cadmium could account for the increased sodium turnover and elevated plasma magnesium concentrations I observed. Likely, therefore, gill electrolyte transport mechanisms are not adversely affected by cadmium.

One of the statistically most significant effects of exposure to 6.4 μg Cd/L was observed in the EOF, that fraction of plasma osmolality accounted for by the sum of the measured electrolyte concentrations. Calculation of the EOF assumes complete ionic dissociation of the electrolytes, which is not absolutely correct since a 135 mmol/L aqueous solution of NaCl has an osmolality of 252 mosmol/kg (Weast 1970). In view of the restricted range in concentration of the various plasma electrolytes I measured, the relative error in the EOF calculation should be uniform across all treatment–time combinations. The average decrease in EOF observed in trout exposed to 6.4 μg Cd/L was 2.7%, which is equivalent to appoximately 8 mosmol/kg. Neither bicarbonate nor phosphate ions increased in the plasma of trout exposed to similar concentrations of cadmium (M. A. Giles, unpubl. obs.) to levels sufficient to account for a significant portion of this unidentified osmolality. Recent observations in our laboratory, however, have demonstrated that rainbow trout exposed to acid stress may elevate blood glucose from control levels of approximately 50 mg/dL to 350 mg/dL (Brown et al. 1984; M. A. Giles, unpubl. obs.). Assuming the glucose is osmotically active these concentrations would represent approximately 2.8 and 19.4 mosmol/kg respectively. A hyperglycemic response, therefore, is more than adequate to account for the unidentified osmotic constituent observed in the plasma of cadmium-exposed trout in my study. Hyperglycemia is a common response to stress in freshwater fish and may serve as an indicator of sublethal environmental pollution under a range of conditions (Chauvin and Young 1970; Silbergeld 1974; Hattingh 1976).

Although increased urinary excretion of sodium was observed in fish exposed to 6.4 μg Cd/L, the ability of the kidney to reabsorb calcium and magnesium was not impaired. In fact, assuming similar glomerular filtration rates in treated and control fish, the absorption of these electrolytes was enhanced in cadmium-exposed fish, presumably as a homeostatic response to decreased plasma calcium concentrations. Such a mechanism, however, is not appropriate for magnesium which was elevated in the plasma of fish exposed to 6.4 μg Cd/L. My results do not support the speculation of Roch and Maly (1979) that the plasma hypocalcemia induced by cadmium is a result of kidney dysfunction. Since trout normally excrete approximately 30–50% of the calcium present in the plasma each day, the decline in plasma calcium (14% less than control values) of fish exposed to 6.4 μg Cd/L may indicate a slight impairment in the mechanisms responsible for calcium influx.

Gill permeability to water, as reflected by the volume of urine excreted, was unaffected by exposure to cadmium. Estimates vary substantially, but about 70–90% of the net osmotic gain of water in freshwater teleosts occurs through the gill epithelium (Maetz 1974). Although the rate of uptake of water by fish may be modified by changes in gill permeability and water drinking rates (Maetz 1974), the net water gain can be balanced only through urinary excretion. The rate of urine production is influenced markedly by handling, anesthetization, and surgery (Hunn and Willford 1970) and by changes in activity and metabolic rate (Wood and Randall 1973; Hofmann and Butler 1979) or dissolved oxygen concentration (Hunn 1969). These effects were minimized in my study because starved fish were allowed a 3-d period of recovery from surgical trauma prior to the 3-d period in which all urinary production was collected for analysis. The daily weight-specific rates of urine production (equation 1) are generally comparable with published values for freshwater trout at a specified weight (Hickman and Trump 1969).

The relatively small plasma electrolyte changes, the maintenance of water permeability relationships, and the apparent competence of kidney function in the trout exposed to cadmium support the suggestion of McCarty and Houston (1976) that sublethal concentrations of cadmium result in a shift of water and electrolytes from the extracellular to the intracellular fluid spaces. The biochemical mechanisms responsible for the cadmium-induced redistribution of electrolytes remain unidentified. Possibly these changes in the chemical composition of the intracellular fluid of the musculature are related to the hyperactivity observed in other freshwater fish exposed to cadmium (Benoit et al. 1976; Ellgaard et al. 1978). Wood and Randall (1973) observed a 10% increase in the concentration of sodium in the epaxial muscle of exercised rainbow trout compared with unexercised controls or exercised fish after a 1-h postexercise recovery.

Many of the responses to cadmium reported here, especially those relating to kidney function, and by Majewski and Giles (1981) were of greatest magnitude during the earlier periods of exposure. This observation suggests that a process of acclimation or detoxification such as the metallothionien response

described by Klaverkamp et al. (1984) for cadmium-exposed white suckers (*Catostomus commersoni*) may have occurred during the extended periods of exposure. The steep slope of the dose–response relationship for cadmium in rainbow trout (no effect at 3.4 μg/L; incipient lethal concentration of 16 μg/L) and the high concentrations of cadmium reported in several aquatic systems (Flick et al. 1971; Henriksen and Wright 1978; McFarlane and Franzin 1978; Murphy et al. 1978) probably indicate that cadmium is now a significant environmental stressor to freshwater salmonids. Although it is likely that some degree of tolerance to this metal can be developed, the concentration limits for acclimation and the metabolic and physiological costs of this stress require definition.

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Osmoregulatory and Hematological Responses of Rainbow Trout (*Salmo gairdneri*) to Extended Environmental Acidification

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The dose-dependent relationships of several physiological responses to acid were examined in rainbow trout (*Salmo gairdneri*) exposed for 22 d to water at pH 4.2–6.0. Significant increases in ventilatory and cardiac rates occurred at $pH_w < 4.9$. Hematocrit and hemoglobin concentration increased progressively with acid stress at $pH_w < 5.5$. Plasma calcium, magnesium, sodium, and chloride concentrations were reduced and plasma phosphate elevated in acid-exposed fish. Seasonal differences were observed in the quantitative responses to acid exposure in the relationship between hematocrit and hemoglobin concentration and in the plasma concentrations of calcium and magnesium. The fractional contribution of sodium and chloride to plasma osmolality decreased linearly with increasing hydrogen ion concentration, and the change in plasma sodium, chloride, and osmolality per unit change in pH_w was 35.2 mmol/L, 39.7 mmol/L, and 47.4 mosmol/kg, respectively, in the pH range of 4.5–5.2. We concluded that the discrepancy between the reduction in plasma osmotic pressure and the combined reduction in the major plasma electrolytes is a result of the elevation in concentration of an unidentified plasma solute which offsets 40–45% of the expected reduction in osmotic pressure.

Nous avons étudié diverses réactions physiologiques reliées au degré d'acidité du milieu chez la truite arc-en-ciel (*Salmo gairdneri*) placée pendant 22 jours dans de l'eau dont le pH se situait entre 4,2 et 6,0. Nous avons noté une augmentation significative du rythme cardiaque et respiratoire lorsque le pH_w était inférieur à 4,9. L'hématocrite et l'hémoglobine augmentaient progressivement avec l'acidité lorsque le pH_w était inférieur à 5,5. Chez les poissons placés en eau acide, la concentration plasmatique de calcium, magnésium, sodium et chlorure était réduite, tandis que celle du phosphate augmentait. Au point de vue quantitatif, la réaction à l'acidité présentait des différences saisonnières que reflétaient la relation entre l'hématocrite et la concentration d'hémoglobine ainsi que la concentration plasmatique de calcium et de magnésium. La part de l'osmolalité plasmatique que représentaient le sodium et le chlorure décroissait de façon linéaire à mesure que la concentration d'ions d'hydrogène augmentait et la variation de l'osmolalité et de la concentration plasmatique de sodium et de chlorure par unité de pH_w était respectivement de 47,4 mosmol/kg, 35,2 mmol/L et 39,7 mmol/L entre un pH de 4,5 et 5,2. Nous avons conclu que la différence entre la baisse de la pression osmotique plasmatique et la diminution de la concentration des principaux électrolytes plasmatiques qui l'accompagne est due à l'augmentation de la concentration d'une substance inconnue soluble dans le plasma dont l'action annule entre 40 et 50 % de la baisse de pression osmotique prévue.

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The acidification of poorly buffered freshwater systems has become the focus of an intensive research effort over the past decade. The loss or reduction of fish populations has been correlated with a decline in the pH of their habitat in a number of studies from Canada, northeastern United States, and Scandinavia (Beamish and Harvey 1972; Schofield 1976; Brown and Sadler 1981). Furthermore, a large number of lakes and streams in southcentral Ontario, in which fish populations are dominated by lake trout, *Salvelinus namaycush*, or brook trout, *Salvelinus fontinalis* (Minns 1981), may soon experience substantial declines in pH (Dillon et al. 1978).

Acute exposures of fish to highly acidic conditions (pH 4.0–4.5) cause impairment of blood acid–base balance (Dively et al. 1977; Neville 1979a; McDonald et al. 1980; Ultsch et al. 1981) and plasma electrolyte regulation (Packer and Dunson 1970, 1972; McWilliams and Potts 1978; Neville 1979b; McDonald et al. 1980); these are the major physiological effects of acid stress. Environmental calcium (Brown 1981; McWilliams 1982a; McDonald 1983; McDonald et al. 1980) and free carbon dioxide concentrations (Lloyd and Jordan 1964; Neville 1979b) appear to modulate the effects of acid. Cardiovascular–respiratory responses such as increased hematocrit or hemoglobin, elevated heart rate, and dorsal aortic blood pressure (Milligan and Wood 1982) and reduced scope for activity, standard metabolic rate, and oxygen consumption during swimming (Waiwood and Beamish 1978) have also been reported at sublethal levels of acid stress.

Most physiological studies have employed short-term exposures to relatively constant highly acidic conditions to identify the responses of the fish to this stress. In many instances

the hydrogen ion concentrations employed approached levels that would be lethal after longer periods of exposure (Spry et al. 1981). Furthermore, some salmonids have the capacity to acclimate to some degree of acid stress (McWilliams 1980, 1982b), which may affect the extrapolation of results from acute studies to more environmentally realistic situations. Poorly buffered aquatic systems subjected to acid deposition commonly exhibit substantial temporal variations in pH. Although the pH of streams in acid-sensitive watersheds may decline by as much as 1 unit during periods of heavy rainfall (Seip and Tollan 1978; Scheider et al. 1979; Haines 1981), the input of acid during snowmelt is probably more significant in most temperate areas (Beamish and Harvey 1972; Hagen and Langeland 1973). The period of snowmelt will depend upon a variety of geological, climatological, and biological factors but 2–6 wk may be representative of many acid-sensitive areas (Galloway et al. 1980; Johannessen et al. 1980). Since fish kills resulting from lowered pH levels during snowmelt have been reported (Leivestad and Muniz 1976), there is a need to identify the dose–response relationships for the major physiological responses of fish exposed to acid stress for time periods more consistent with the duration of snowmelt. Therefore, we undertook to identify the dose-dependent responses in rainbow trout, *Salmo gairdneri*, exposed to acid stress for 3 wk.

Materials and Methods

Water Acidification and Carbon Dioxide Equilibration

Dechlorinated Winnipeg City water was acidified to pH 6.0 and the excess carbon dioxide removed by aeration in a system consisting of four 325-L reservoirs in series. Each reservoir was equipped with a Radiometer TTT-2 titrator that monitored water pH (pH_w) and controlled the input of 0.9 mol H_2SO_4/L through Radiometer type NMVID valves or a Cole-Parmer model 7016 Masterflex pump. The acidified water in each reservoir was recirculated through three PVC columns (100 cm × 80 cm^2; height × area) containing porcelain gas exchange chips and receiving a counter-current flow of air at 10 L/min in each column. The P_{CO_2} in the final reservoir was 1.1–1.3 mmHg. This water was delivered from a constant head tank to five 175-L fiberglass aquaria at a rate of 2 L/min. The 99% replacement time was approximately 6 h. The pH in each aquarium was controlled by a Radiometer TTT-2 titrator that regulated the addition of 0.9 mol H_2SO_4/L. Water circulation and aeration was maintained with an air-lift pumping system.

The chemical characteristics of Winnipeg City water were determined by standard analytical methods (Stainton et al. 1977) and heavy metals by atomic absorption spectrophotometry. The mean (SD) concentrations (micromoles per litre) were as follows: Na^+, 82.6 (4.3); K^+, 33.3 (2.6); Cl^-, 104.4 (16.9); Ca^{2+}, 540.6 (42.9); Mg^{2+}, 238.4 (31.5); SO_4^{2-}, 67.6 (5.2); total dissolved inorganic carbon, 1650 (210); total dissolved organic carbon, 670 (103); $NH_3 + NH_4^+$, 1.4 (0.8). The concentrations of Cd, Cu, Pb, Ni, and Zn were < 2 μg/L. The inorganic carbon in each experimental tank was measured using a modification of the method of Stainton et al. (1977) in which a 5-mL sample of acidified water was equilibrated with a 5-mL volume of nitrogen in a gas-tight syringe and the concentration of carbon dioxide in the gas phase measured on a gas chromatograph. The gas chromatograph consisted of a 0.5-mL sample loop, a 0.3 × 122 cm (inside diameter × length) column of Porapak Q absorbent (Waters Associates Inc., Framingham, MA) and a Carle model 100 micro-thermal conductivity detector. Helium (12 mL/min) was used as a carrier gas and the sample to be analyzed was dried with magnesium perchlorate prior to injection. This method could detect free carbon dioxide at levels of 2 μmol/L.

Experimental Animals

Immature rainbow trout of either sex, mean weight (SD) of 217.4 g (43.6), were obtained from the Rockwood Experimental Fish Hatchery, Rockwood, Manitoba, and held in dechlorinated water at a 12 h light : 12 h dark photoperiod for at least 2 wk prior to use. Ten or 11 fish were then transferred into the fiberglass aquaria and held for 1 wk in decarbonated water at pH 6.0. The pH-controller on each aquarium was then activated and the desired hydrogen ion concentration achieved within a few minutes. Tanks kept at pH 6.0 served as controls, and in one experiment fish kept at pH 7.5 in untreated water served as an additional control. Two experiments were conducted, the first in December–January and the second in the following March–April. Mean water temperature (SD) was 13.6 (0.3) and 12.5 (0.1)°C during these respective periods. The fish were fed commercial food pellets once daily for 5 d/wk at approximately 2% body weight/d. Fish mortality was monitored hourly for the first 2 d of exposure and three times daily thereafter. After 19 d of exposure in the December–January experiment four fish from each tank were directed into cylindrical (9 cm × 36 cm, inside diameter × length) electrode chambers (Majewski et al. 1979) without removing the fish from the water. Respiratory and cardiac rates were determined from recordings of the biopotentials in the water surrounding the fish through a set of electrodes positioned dorsally and ventrally near the head region and connected to a Hewlett-Packard physiograph (model 7754A) through a bioelectric amplifier (model 8811A). Cardiac and ventilatory rates were monitored twice daily for the following 3 d.

Following the 22-d exposure to acid, the fish were netted, killed with a blow to the head, and bled immediately from the caudal region into heparinized (NH_4-heparin, Sigma Chemical) syringes. The blood was well mixed and samples removed for determination of hematocrit and hemoglobin. Hematocrit was determined by centrifugation at 13 700 *g* for 5 min in microhematocrit tubes; hemoglobin concentration was measured as cyanmethemoglobin at 540 nm with a Bausch & Lomb Spectronic 710. The remaining blood was centrifuged at 8600 × *g* for 2 min and the plasma removed for chemical analyses. In fish from the March–April experiment, a sample of epaxial muscle was removed from the area just anterior to the insertion of the dorsal fin and a subsample of approximately 2 g weighed, dried under 730 mmHg of vacuum at 95°C for 24 h, and reweighed to determine tissue water. Tissue electrolyte concentrations were estimated according to the procedures described by Eddy and Bath (1979) by homogenizing 500 mg of epaxial muscle with 3 mL of distilled water for 30 s in a Willems polytron. Homogenized samples were capped, stored for 4 d at 4°C, and centrifuged at 2000 × *g* for 20 min. The supernatant was frozen until analyzed for the various tissue electrolytes.

Plasma Analyses

Plasma osmolality was measured on duplicate 300-μL samples with a model 3R Advanced Instruments Inc. osmometer. Sodium and potassium levels of plasma and tissue extracts were determined in duplicate with a Beckman KLiNa-Flame

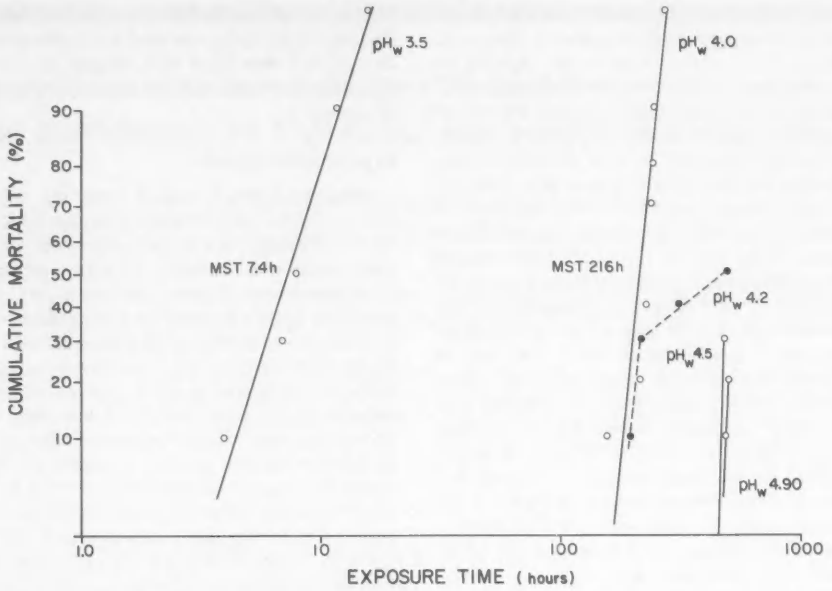

FIG. 1. Survival times of juvenile rainbow trout in acid water over a 22-d exposure period in December–January (○) and March–April (●). MST = median survival time.

photometer. Plasma and tissue chloride was measured in duplicate with a Radiometer CMT-10 chloride titrator and calcium and magnesium were measured by atomic absorption of 422.7 and 285.2 nm, respectively, with a Unicam SP90 spectrophotometer. The concentrations of inorganic phosphate and sulphate in plasma were measured after separation on an ion-exchange column by the method of Stainton et al. (1977). Plasma water concentration was determined by measuring the weight loss upon drying at 95°C under 730 mmHg of vacuum for 24 h on triplicate 500-μL samples of plasma.

Tests of significance of the correlation coefficients from least-square linear regressions and of differences between means were performed using Student's *t*-test.

Results

Fish Mortality

The lethal toxicity of acid water to trout is illustrated in Fig. 1. At pH_w 3.5 and 4.0 all fish died. Mortalities of 50, 30, and 20% were observed within the 22-d exposure period at pH_w 4.2, 4.5, and 4.9, respectively. Although extrapolation of the line for pH_w 4.2 would suggest that complete mortality would occur after approximately 300 d, the steep slope of the mortality–time relationships at lower pH_w values and the extensive loss of blood electrolytes in these survivors (see following sections) would indicate that all the fish at pH_w 4.2 would have died within a few days of the termination of the experiment.

Ventilatory, Cardiac, and Erythrocyte Responses

Ventilaton rate after exposure to pH_w 4.9 and 4.5 increased by 15 and 24%, respectively, over values at pH_w 6.0 (Table 1). Cardiac rate was increased by 74 and 61%, respectively, over control values at pH_w 4.9 and 4.5. Although ventilatory and cardiac rates were not measured in fish at pH 7.5, the control values for both ventilation and cardiac rates at pH_w 6.0 are not significantly different from values observed in unstressed trout in nonacidified water at pH_w 7.8 monitored under similar conditions.

Hematocrit and hemoglobin concentration increased progressively at $pH_w < 5.5$ during both exposure periods (Table 1). At pH_w 4.2, hematocrit and hemoglobin were 58 and 41% higher, respectively, than control values during the same experimental period but ratios of hematocrit to hemoglobin concentration (Hct:Hb) were dissimilar in the two experimental periods. An increase in this ratio would indicate either a decrease in mean cellular hemoglobin of newly released erythrocytes, an increase in volume (swelling) of circulating erythrocytes, or a combination of these factors. Although the Hct:Hb ratio of control fish was slightly lower than that of acid-exposed trout in the December–January experiment the differences were statistically insignificant and there was no relation between the ratio and the degree of acid stress. In the March–April exposures, however, Hct:Hb increased progressively with increasing severity of acid stress at $pH_w < 5.2$ and became significantly higher at pH_w 4.7 and 4.2.

Plasma and Muscle Electrolytes

Plasma calcium (Ca_p) and magnesium (Mg_p) declined in response to decreasing pH but at any particular water pH the concentration of both electrolytes were lower in December–January samples (Fig. 2). In both experiments the decline in Ca_p was reversed at pH_w 4.2–4.5. Possibly, calcium was being mobilized from bone and scales or tissue compartments at the higher levels of acid stress. The maximum acid-induced decreases in Ca_p and Mg_p averaged 23% (21–24%) and 28% (24–32%), respectively, from control values.

The effects of acid stress on Na_p and Cl_p were similar in both experimental periods (Fig. 3). Na_p and Cl_p were unaffected as

TABLE 1. Effect of acid stress on ventilatory and cardiac rates and blood Hct:Hb ratios in rainbow trout susriving 22 d of exposure to acid water. Results are means (1 SD). Significance level for comparisons of treated fish and controls (*t*-test) at pH_w 6.0: $*P < 0.05$; $**P < 0.01$.

pH_w	Pco_2 (mmHg)[a]	Survival (%) (*N*)	Hct (% RBC)	Hb (g/dL)	Hct:Hb (mL/g)	Ventilation rate (cycles/min)	Cardiac rate (beats/min)
Dec.–Jan.							
7.5	0.7–0.8	100 (7)	35.1 (4.4)	9.6 (1.9)	3.73 (0.56)*	62.3 (5.8)[b]	40.4 (4.9)[b]
5.96 (0.11)	2.3–2.4	100 (8)	35.6 (4.7)	8.2 (1.1)	4.33 (0.37)	66.3 (5.7)	35.3 (5.2)
4.90 (0.10)	2.1–2.3	80 (8)	43.4 (5.2)**	9.9 (1.6)	4.38 (0.33)	76.3 (14.8)**	61.5 (12.5)*
4.46 (0.05)	2.2–2.3	70 (7)	45.1 (5.8)**	10.6 (1.6)	4.32 (0.58)	82.0 (4.6)**	56.8 (7.9)**
Mar.–Apr.							
5.96 (0.04)	2.7–2.8	100 (8)	30.0 (7.2)	6.4 (1.4)	4.34 (0.38)		
5.49 (0.04)	2.7–2.7	100 (10)	37.6 (7.1)*	7.7 (1.2)	4.90 (0.27)**		
5.20 (0.08)	2.5–2.7	100 (11)	39.1 (4.4)**	8.1 (1.0)*	4.86 (0.50)**		
4.72 (0.14)	2.4–2.5	100 (11)	40.0 (5.5)**	7.8 (1.0)*	5.11 (0.26)**		
4.21 (0.08)	2.0–2.1	50 (4)	47.3 (2.8)**	9.0 (0.4)**	5.23 (0.09)**		

[a]Range of Pco_2 observed over experimental period.
[b]Ventilatory and cardiac rates at pH 7.8 from Majewski and Giles (1981).

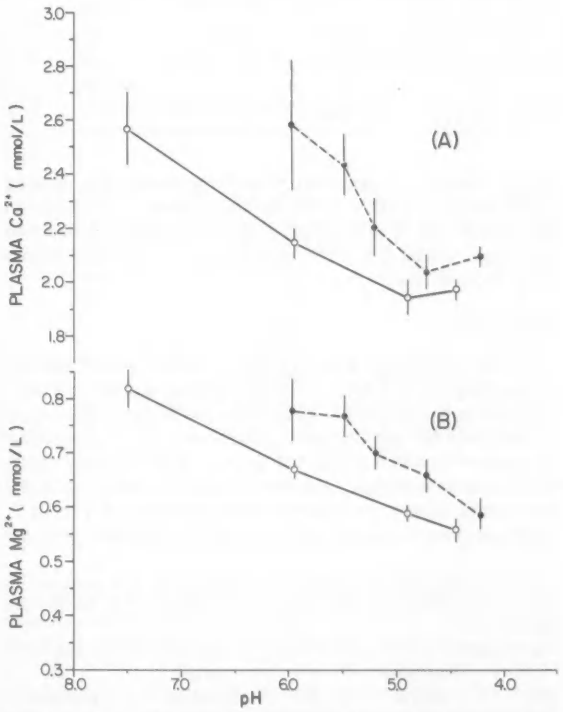

FIG. 2. Effect of water pH upon total plasma (A) calcium and (B) magnesium concentrations in trout exposed for 22 d (mean ± 1 SE). Exposure periods: ○, December–January; ●, March–April.

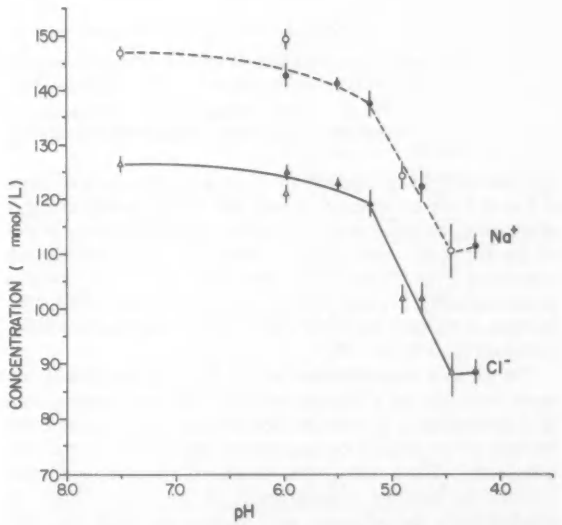

FIG. 3. Effect of water pH upon plasma sodium and chloride concentrations in trout exposed for 22 d (mean ± 1 SE). Exposure periods: ○, December–January; ●, March –April.

pH_w was decreased from 7.5 to 5.2; however, as pH_w was decreased from 5.2 to 4.5, the concentrations of both ions decreased in a linear manner such that $\Delta Na_p/\Delta pH_w$ and $\Delta Cl_p/\Delta pH_w$ were 35.2 and 39.7, respectively. These slopes were not significantly different ($t = 0.024$, $P < 0.5$, df = 64), indicating that plasma losses of both ions were equivalent in this pH range. A reduction in pH_w from 4.5 to 4.2 did not result in a further decrease in Na_p and Cl_p. Analysis of tissue electrolyte levels (following section) demonstrated that at pH_w 4.2, tissue sodium was significantly reduced, and it is probable, therefore, that tissue sodium was contributing to the maintenance of plasma sodium at this level of stress.

Plasma osmolality (O_p) declined by less than 2.5 mosmol/kg as pH_w was reduced from 7.5 to 5.5 (Fig. 4). Between pH_w 5.2 and 4.5, however, O_p declined in an approximately linear manner such that $\Delta O_p/\Delta pH_w$ was 47.4. As pH_w was reduced from 4.5 to 4.2, O_p rose by approximately 19 mosmol/kg. The decrease in O_p as pH_w was reduced from 5.2 to 4.5 was 35.1 mosmol/kg, which is 63% of the combined reduction in Na_p and Cl_p observed in this pH range. Assuming complete ionic dissociation, Na_p and Cl_p together can account for 92–93% of the osmolality of plasma in control trout. Thus, the comparatively small reduction in O_p as pH_w declined from 5.2 to 4.5 and

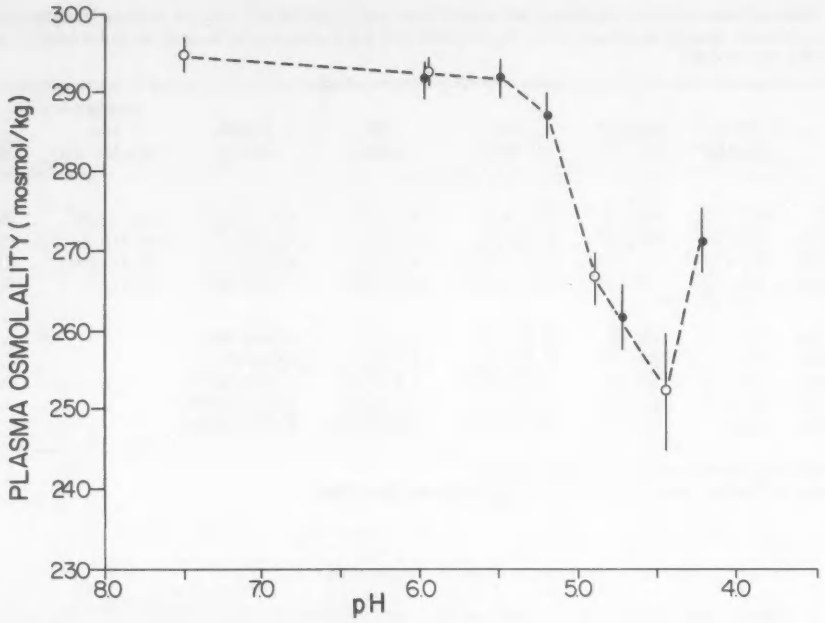

FIG. 4. Effect of water pH upon plasma osmolality in trout exposed for 22 d (mean ± 1 SE). Exposure periods: ○, December–January; ●, March–April.

the relatively sharp increase in O_p as pH_w was reduced from 4.5 to 4.2 are not consistent with the corresponding changes observed in the major osmotic constituents of the plasma. A plot of the fractional contribution of Na_p plus Cl_p to the plasma osmolality (Na+Cl/osmolality) over the range of pH_w tested is presented in Fig. 5. Clearly, as pH_w decreases from 6.0 to 4.2 the fraction of the osmolality not attributable to Na+Cl/osmolality increases from 10 to 27%.

The plasma concentrations of sulphate and phosphate ion were measured on a limited number of frozen samples from acid-stressed trout in order to identify their contribution to the fraction of the plasma osmolality not attributable to Na+Cl/osmolality. These ions were measured because a 12-fold increase in sulphate concentration of test water (Table 1) resulted from the sulphuric acid required to titrate the 1650 μmol of bicarbonate ion present in the laboratory water supply, and changes in plasma phosphate could result from reabsorption of calcium phosphate from bone. No relationship between pH_w and sulphate concentration in plasma was observed (Table 2). Plasma sulphate concentration was approximately 10-fold greater than the sulphate concentration in nonacidified water at pH 7.5 and approximately equal to the concentration in acidified water. Plasma phosphate concentrations increased with increasing severity of acid stress (Table 2). Although considerable variability was observed in plasma phosphate a least squares regression of all values at $pH_w \leq 6$ yielded $[PO_4]$ mmol/L = $3.96 - 0.25\ pH_w$ for which $r = 0.53$ ($P \leq 0.01$). The acid-induced increase in plasma phosphate, however, could account for less than 2% of the non-(Na+Cl/osmolality).

Acid exposure had no effect upon muscle calcium, magnesium, chloride, or water concentrations (Table 3). The lack of effect upon muscle water was surprising, since at pH_w 4.2 plasma water was significantly ($P < 0.01$) reduced. Muscle sodium was reduced significantly ($P < 0.05$) by 20 and 30% at pH_w 4.7 and 4.2, respectively. Muscle potassium was reduced by 6% at pH_w 4.7 ($P < 0.10$) but was elevated at pH_w 4.2, an observation that is anomalous in that the mean included two very high values (147–150 mmol/kg) and two low values (113–115 mmol/kg).

Moribund Fish

Moribund fish were characterized by lack of orientation and surface gulping, and fish exhibiting similar characteristics were observed to die within 12–14 h. Some blood characteristics of 10 moribund fish are compared with those of fish surviving the 22-d exposure period at pH_w 4.2 and 4.5 (Table 4). Some dying fish exhibited extremely elevated hematocrits although many had values similar to those in fish surviving at pH_w 4.2. Although plasma calcium was depressed in moribund trout the

TABLE 2. Inorganic phosphate and sulphate ion concentrations in plasma of rainbow trout exposed to acid stress for 22 d. Results are means (1 SD).

pH_w	Exposure period	N	PO_4 (mmol/L)	SO_4 (mmol/L)
4.21	Mar.–Apr.	1	3.56	0.69
4.46	Dec.–Jan.	2	(2.72–3.58)[a]	(0.73–0.78)[a]
4.72	Mar.–Apr.	5	2.98 (0.22)	0.55 (0.06)
4.90	Dec.–Jan.	3	2.47 (0.40)	0.65 (0.09)
5.20	Mar.–Apr.	3	2.72 (0.33)	0.74 (0.11)
5.49	Mar.–Apr.	5	2.59 (0.37)	0.87 (0.12)
5.96	Dec.–Jan.	3	2.31 (0.10)	0.59 (0.23)
5.97	Mar.–Apr.	3	2.50 (0.28)	0.67 (0.13)
7.5[b]		6	2.77 (0.36)	0.51 (0.12)

[a]Range given for $N = 2$.
[b]Untreated controls in nondecarbonated water.

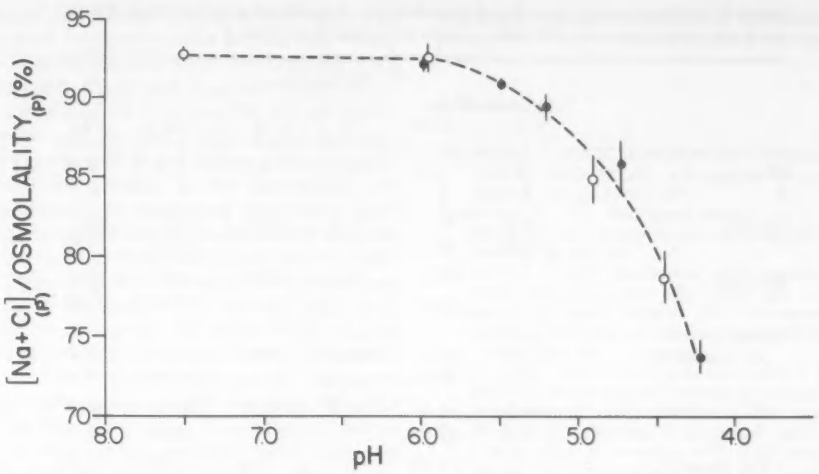

FIG. 5. Effect of water pH on the fractional contribution of sodium and chloride to the plasma osmolality in trout exposed for 22 d (mean ± 1 SE). Exposure periods: ○, December–January; ●, March–April.

TABLE 3. Effect of 22-d exposure to reduced pH on tissue water and electrolytes in rainbow trout. Results are means (1 SD) for exposures during March–April expressed per wet weight of tissue. Significance level for comparisons of treated fish and controls (*t*-test): $*P < 0.05$; $**P < 0.01$.

pH_w	N	Na^+ (mmol/kg)	K^+ (mmol/kg)	Ca^{2+} (mmol/kg)	Mg^{2+} (mmol/kg)	Cl^- (mmol/kg)	Tissue water (%)	Plasma water (%)
4.21	4	8.09**	131.3	1.99	9.05	20.5	78.8	91.9**
		(0.95)	(20.0)	(0.74)	(1.21)	(5.2)	(0.9)	(1.0)
4.72	11	9.15**	115.5*	1.88	9.05	19.2	78.4	94.2
		(2.05)	(8.9)	(4.5)	(0.93)	(3.2)	(1.4)	(2.1)
5.20	11	10.65	125.1	1.82	9.04	21.8	77.7	94.4
		(1.30)	(9.1)	(3.6)	(1.80)	(3.7)	(1.0)	(1.0)
5.49	10	10.49	121.3	1.65	8.50	22.0	78.0	94.6
		(0.72)	(±2.3)	(0.36)	(1.59)	(1.8)	(0.8)	(0.4)
5.97	11	11.34	122.1	1.86	9.74	20.5	78.8	94.7
		(1.55)	(6.2)	(0.24)[a]	(0.89)[a]	(3.2)	(1.8)	(1.6)

[a]Sample size 10.

most obvious characteristic was the loss of plasma sodium and chloride, which ranged from 54 to 60 and 44 to 50%, respectively, of values observed in control fish or fish exposed to acid at $pH_w > 5.5$ (Fig. 4). This loss of sodium and chloride regulation was reflected directly in the plasma osmolality, since the (Na+Cl/osmolality) was generally similar in moribund fish and those surviving at pH_w 4.2 and 4.5 for 22 d.

Discussion

Response thresholds to chronic acid exposure were observed in all variables with the exception of tissue calcium, magnesium, chloride, and water concentrations and plasma sulphate. From visual inspection we ranked the thresholds in order of decreasing sensitivity to acid stress as follows: Ca_p = $(Na+Cl/osmolality)_p$ = Hct > O_p = PO_{4p} = Mg_p > Na_p = Cl_p = Hb > tissue Na > plasma water. Insufficient data were obtained to rank the cardiac and respiratory rates. Although these ranks may reflect the sensitivity of each variable to acid, the usefulness of a physiological response as an indicator of the level of stress experienced by a population of fish will be influenced by its inherent variability and systematic changes in variability over the response range. The slope of the dose–response relationship will govern the sensitivity in detecting small variations in stress, and changes in response threshold resulting from previous exposure history or seasonal factors may also influence its applicability. For example, while we observed reasonable dose–response relationships between acid stress and Hct, Ca_p, Mg_p, and PO_{4p}, the responses were quantitatively or qualitatively different during the two exposure periods. Plasma sodium, chloride, and osmolality exhibited relatively sharp response thresholds at pH 5.2–5.5 but also exhibited a reversal in response at pH 4.2 and an increase in variance with increasing levels of acid stress.

TABLE 4. Comparison of some blood characteristics of moribund trout with those that survived the highest levels of acid stress. Results are means (1 SD) when $N > 3$.

	Survival time (d)					
	9–11	8–9	13	21	>22[a]	>22[a]
pH_w	4.01	4.21	4.21	4.21	4.21	4.46
N	5	3	1	1	4	6
Hct (%)	59.5 (10.9)	49.2 (5.3)	71.0	60.0	47.3 (2.8)	45.1 (5.8)
Hb (g/dL)		9.9 (1.8)	11.8	10.5	9.0 (0.4)	10.6 (1.6)
Plasma						
Na^+ (mmol/L)	84.7 (7.3)	82.6 (9.6)	77.8	73.1	111.5 (4.6)	110.6 (11.2)
Cl^- (mmol/L)	61.1 (7.0)	65.0 (7.9)	55.5	55.0	88.5 (5.1)	88.3 (9.5)
Ca^+ (mmol/L)		1.56 (0.18)		1.62	2.10 (0.08)	1.98 (0.09)
Mg^{2+} (mmol/L)		0.42 (0.05)		0.33	0.59 (0.07)	0.56 (0.05)
Osmolality (mosmol/kg)	190.8 (10.7)	187.9 (12.8)	194.0	196.3	271.1 (8.4)	252.1 (16.3)
Na + Cl/osmolality (%)	76.1 (4.7)	78.5 (5.7)	68.7	65.3	73.8 (2.0)	78.7 (3.9)

[a]Nonmoribund survivors.

The most consistent response to acid stress was observed in the Na+Cl/osmolality fraction of plasma, which exhibited a continuous decline with increasing hydrogen ion concentration at pH < 6 and a low, relatively uniform variance (coefficient of variation: average 3.2, range 0.7–6.8%) over the entire pH range examined. The Na+Cl/osmolality fraction of plasma, hereafter denoted as OF_p, is a composite parameter that segregates the contribution of sodium and chloride to the total osmotic pressure from the contribution of all other electrolytes and organic molecules. The OF_p of unstressed rainbow trout was 92.4%, similar to the value of 91% observed in brown trout, *Salmo trutta*, (Fugelli and Vislie 1982). At physiological concentrations, 1 mmol NaCl/L is equivalent to 1.86 mosmol/kg (Weast 1970). The actual contribution of sodium plus chloride to the plasma osmotic pressure can be calculated, therefore, as 89.2% on the basis of control data in this experiment. A reduction OF_p represents a proportional increase in the concentration of one or more of the constituents that contribute to the remaining 10–11% of the plasma osmotic pressure. At pH 4.5 we observed a 37-mmol/L decrease in both Cl_p and Na_p whereas O_p decreased by only 42.5 mosmol/kg. Thus, approximately 29.5 mosmol/kg or 40% of the expected decline in osmotic pressure was compensated for by a rise in some constituent in the plasma. Fugelli and Vislie (1982) reported a 52.4-mosmol/kg decrease in plasma osmolality for the same decrease in plasma sodium plus chloride in brown trout exposed to pH 4.0–4.6 for periods up to 20 d. This represented a 27% compensation of the expected decrease in osmolality. In our study this osmotic compensation was evident in the pH range of 5.5–4.2, inclusive.

The identity of the solute responsible for the acid-induced change in OF_p was not identified but it is probable that elevated glucose levels were involved. Severe hypoglycemia has been reported in acid-exposed rainbow trout (Lee et al. 1983; McDonald 1983; Brown et al. 1984) and the response appears to be dose dependent. This may be a generalized response to reduced blood electrolyte concentrations, since Bergstrom (1971) reported a linear inverse relationship between plasma sodium chloride and glucose concentrations in Atlantic salmon, *Salmo salar*, during prolonged exposure to distilled water. In that study, glucose increased by 1 mol for each 3.4-mol reduction in plasma sodium chloride. Although elevations of 4.2 mmol/L have been reported in the plasma amino acids of chronically acid-stressed trout (Fugelli and Vislie 1982), these changes could account for less than 10% of the change in OF_p. Alternatively, McDonald (1983) reported a 7-mmol/L increase in plasma amino acids in rainbow trout after acute (< 4 d) exposure, which could offset 14% of the expected decline in OF_p. Hyperglycemia is a sensitive indicator of environmental and handling stress (Hattingh 1976; Silbergeld 1974) but this response is of far lesser magnitude than that involved in the osmotic compensation observed in acid-stressed trout. The question of whether the hyperglycemic response is a controlled mechanism for osmotic compensation during plasma demineralization or a secondary consequence of impaired neuroendocrinological and metabolic function remains unanswered.

A comparison of sodium efflux rates reported for acute exposures to acidic water with those calculated from the change in plasma sodium concentration we observed after 21 d of acid exposure indicates that the rate of sodium loss is reduced during extended acid stress. Net branchial efflux rates for sodium at a pH_w of approximately 4.0–4.2 have been reported as 131 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for brown trout (McWilliams 1980). An additional 20–28 $\mu mol\ Na \cdot kg^{-1} \cdot h^{-1}$ may be lost via renal

excretion (McDonald and Wood 1981). Using total body sodium values reported for rainbow trout in freshwater (Eddy and Bath 1979) of 52 mmol/kg body water, the foregoing efflux rates represent losses of 6–8% per day of the total body sodium. Clearly, these rates are excessive in terms of the average rates of sodium loss observed over the 22-d period of acid exposure. Assuming that changes in muscle and plasma sodium concentrations reflect respective changes in the intracellular and extracellular compartments, an estimate of total body sodium loss and the average net efflux rate can be calculated using the extracellular and intracellular fluid volumes reported by Eddy and Bath (1979). The average net effluxes thus calculated were 0.3 and 1.2% per day of total body sodium for trout held at pH_w of 5.2, 4.7, and 4.2, respectively. The values at pH_w 4.2 are only 30% of those observed in acute exposures by McDonald and Wood (1981) and McWilliams (1980) under comparable conditions of water composition and pH. Although the efflux rates calculated for this study may be relatively constant throughout the exposure period, possibly some degree of acclimation occurred at later stages of exposure. More likely, however, a degree of acclimation occurred during the 1-wk period prior to acid exposure when the fish were held in decarbonated water at pH 6.0. McWilliams (1980) has shown that a 6-wk period of exposure to pH 6 decreases the rate of sodium loss in brown trout subsequently exposed to more acid conditions. Interestingly, the elevations in hematocrit, hemaglobin concentration, and heart rate we observed are similar in magnitude to those observed during acute exposures of various salmonids to acidic water (Dively et al. 1977; Neville 1979a; Milligan and Wood 1982; Nieminen et al. 1982). Possibly, these responses are less readily adjusted during prolonged acid stress than those involving ionoregulation.

Although our investigation was not designed as an acute lethality study, it is clear that the rainbow trout were considerably more resistant to acidified water than those employed by previous workers (Lloyd and Jordan 1964; Kwain 1975; Graham and Wood 1981) in which the 4- to 7-d LC50 for trout weighing 1–10 g ranged from 3.9 to 4.3. We observed a median survival time of 216 h at pH 4.0 whereas at pH 4.2, 50% of the fish survived more than 500 h. This increase in resistance probably reflects the larger size of trout (217 ± 43.6 g), since Lloyd and Jordan (1964) observed a positive correlation between survival time at pH 3.6 and age (and presumably size).

We conclude that rainbow trout exposed to water at $pH \leq 4.9$ for periods that approximate that of snowmelt in temperate regions of North America may be expected to experience a substantial impairment of plasma electrolyte regulation and osmotic balance and exhibit various hematological and cardiovascular abnormalities. After exceeding a pH threshold the magnitude of the responses were generally proportional to the level of acid stress. Lee et al. (1983) reported somewhat similar results in rainbow trout exposed to acid stress for 2 or 3 wk and suggested a pH_w of 5.0 as a "lower limit of safety" for this species. From our results we suggest that this limit may occur at a pH_w between 5.2 and 5.5 in terms of the regulation of major plasma electrolytes and osmotic pressure although other indications of stress may be observed at pH_w 5.5–6.0. Based on the severity of ionoregulatory impairment, it is unlikely that rainbow trout would survive continuous exposure to $pH_w < 4.7$.

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Changes in Populations and Drift of Stream Invertebrates Following Lampricide Treatment

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The effects of 3-trifluoromethyl-4-nitrophenol (TFM) treatment on invertebrate abundance, recolonization, and drift rates were examined in three Great Lakes tributaries, having a wide range of water hardness. In the softwater streams only philopotamid caddisflies and lumbriculid worms were significantly reduced after the TFM treatment, whereas their abundance in the untreated stream sections was unchanged. Treatment of the hardwater stream resulted in a dramatic mortality of leeches. Abundance of soft-bodied oligochaetes and Turbellaria were significantly reduced, while numbers of Simuliidae, *Hydropsyche*, and *Baetis* increased 4 d after treatment. Daytime drift rates of the Annelida, Turbellaria, Amphipoda, Glossosomatidae, and Tipulidae increased significantly during the lampricide treatment. Increased drift rates rather than mortality appears to be the major impact of the lampricide on invertebrate communities.

Nous avons examiné les effets (abondance, recolonisation, dérive) sur les invertébrés du traitement au 3-trifluorométhyl-4-nitrophénol (TFM) dans trois affluents des Grands Lacs ayant des eaux de dureté variable. Dans les cours d'eau où les eaux étaient douces, seuls les phryganes Philopotamidae et les vers Lumbriculidae voyaient leur nombre réduit par le traitement au TFM, alors que leur abondance dans les eaux non traitées ne variait pas. Dans les eaux dures la mortalité des sangsues était considérable. L'abondance des oligochètes et des Turbellaria était réduite de façon significative, alors que les Simuliidae, *Hydropsyche* et *Baetis* étaient plus nombreux 4 jours après le traitement. La dérive diurne des Annelida, Turbellaria, Amphipoda, Glossosomatidae et Tipulidae augmentait de façon significative pendant le traitement lampricide. L'effet principal du traitement lampricide sur les invertébrés semble être une augmentation de la dérive plutôt que de la mortalité.

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The most successful method of controlling numbers of sea lamprey (*Petromyzon marinus*) in the Great Lakes has been through use of the selective chemical 3-trifluoromethyl-4-nitrophenol (TFM). Over 250 streams throughout the Great Lakes watershed are regularly treated by agents of the Great Lakes Fisheries Commission. Ammocoetes inhabit the tributaries for several years prior to transforming into parasitic adults; so, each stream is normally treated once every 3 or 4 yr. Exposure during treatment is less than 24 h, and the chemical is not persistent (Carey and Fox 1981). A small amount of the molluscicide Bayer 73 (2′,5- dichloro-4′-nitrosalicylanilide) is sometimes added as a synergist, reducing the amount of TFM required for effective treatment (Howell et al. 1964).

As with most chemicals, TFM affects nontarget organisms to varying degrees. Toxicity varies with water hardness, pH, and temperature, as well as the physiological state of the organism (Marking and Olsen 1975). The concentration of TFM applied varies from stream to stream and is calculated from bioassays performed on lamprey ammocoetes and rainbow trout. Treatments are less than 3 $mg \cdot L^{-1}$ in softwater tributaries and normally 5–10 $mg \cdot L^{-1}$ in hardwater tributaries of the Great Lakes (Gilderhus and Johnson 1980).

Concerns about the impacts of repeated treatments on stream communities, especially in productive trout streams, have been expressed to the Great Lakes Fisheries Commission (1981 Exec. Rep.). Several laboratory bioassays of TFM on aquatic biota have been made (Smith 1967; Maki et al. 1975; Rye and King 1976). The previous field surveys on TFM-treated streams (Torblaa 1968) were not statistically adequate due to low numbers of replicate samples. No studies have examined the effects of treatment on invertebrate drift. The present study was designed to determine the populations, drift, and colonization of invertebrates following lampricide treatment of streams with widely different water hardness.

Locations and Treatments

Two of the streams examined were softwater tributaries of Lake Superior, and the other was a hardwater stream entering Lake Ontario. The Goulais River and its tributary, Achigan Creek, are softwater streams with alkalinities of 12–20 meq. The streams flow through remote, mixed forest on the Canadian Shield, north of Sault Ste. Marie, Ontario. The Goulais system has been treated seven times previously beginning in 1960. All the treatments except 1964 began from Whitman Falls, up-

TABLE 1. Summary of stream characteristics at each study site on the sample dates. AP = application point.

Stream	Stn.	Temp. (°C)	Width (m)	Discharge ($m^3 \cdot s^{-1}$)	Depth (cm)	Current ($cm \cdot s^{-1}$)	Distance below AP (km)
Soper Creek	1	7	7	1.47	27	100	1.5
		5	6.5	1.13	23	97	
	Drift	6–7	9	1.50	32	60	
		4–9	9	1.16	28	58	1.7
		4–8	9	1.13	27	59	
	2	8	6.9	1.50	28	110	2.2
		8	6.3	1.14	24	91	
Achigan Creek	3	14	9.5	1.32	20	78	−3.3
		18	—	—	17	62	
	2	16	9	1.50	25	83	6.0
		21	—	—	23	80	
	1	—	8.5	1.61	21	98	6.3
		22	—	—	20	100	
Goulais River	X	15	39	8.28	22	104	−1.5
		22	—	—	20	72	
	B	—	36	8.22	30	74	7.3
		23	—	—	23	62	
	A	15	43	9.70	23	100	11.2
		22	—	—	21	84	

stream of Searchmount, Ontario. Two riffles were sampled below the application point (Table 1). The untreated, control riffle (stn. X) was 1.5 km upstream of the application point and had been treated only during 1964.

Achigan Creek has been treated from below the village of Wabos on the same dates as the Goulais River. Two riffles (Table 1) were sampled below the application point. The riffle that had never been treated (stn. 3) was 3.3 km above the application point. Treated and untreated sites in each stream were chosen on the basis of similar velocity, depth, and gravel size. Bioassays indicated that minimum lethal concentrations of 1.1 and 0.8 $mg \cdot L^{-1}$ for 12 h in the Goulais River and Achigan Creek, respectively, ensured complete mortality of the ammocoetes. The 1982 treatment was done June 14–15, with an application of 1.1–2.0 mg TFM $\cdot L^{-1}$ in the main river and 1.3–1.9 $mg \cdot L^{-1}$ in Achigan Creek for 20 h.

Soper Creek, like most tributaries of Lake Ontario, is hard, with an alkalinity of 230 meq. The watershed north of Bowmanville, Ontario, is partly wooded with mixed agriculture on drumlinized limestone till (Webber and Morwick 1946). Soper Creek had been previously treated from a small dam four times between 1971 and 1980. The 1983 application point was 1.7 km upstream of the former application point. Bioassay of the lampricide for a 12-h exposure indicated that a minimum concentration of 5.2 $mg \cdot L^{-1}$ was lethal to lamprey ammocoetes. Treatment on May 6, 1983, was a mixture of TFM with 0.8% Bayer 73 (TFM-2B) at a mean concentration of 6.7 $mg \cdot L^{-1}$ maintained for 13 h. Two dissimilar riffles below the 1983 application point were sampled 3 d prior to the treatment and again 4 d after.

The upper riffle, above the former application point, had never been previously treated. This site had a fast flow over gravel and cobble, as do most streams along the north shore of Lake Ontario. A second riffle (stn. 2) was 0.5 km downstream of the former application point, on poorly sorted gravel and sand, immediately below a large pool. This area had a large population of oligochaetes that are known to be sensitive to the lampricide (Gilderhus and Johnson 1980). Drift samples were collected at a third site, 70 m downstream of the small dam used as the former application point.

Methods

Riffle samples were collected using a 500-μm Surber sampler (920 cm^2) in midstream along longitudinal lines where depth, flow, and sediment size were similar. During sampling, each location was marked with a coloured peg to enable quick reference to these variables. When samples were to be retaken within a week, the markers were left in the stream to prevent resampling the same spot.

Rocks inside the sampler, including those with attached algae or moss, were removed to a screen-bottomed bucket. The streambed inside the frame was dug up to a depth of 12 cm with a small trowel. The rocks were inspected, attached vegetation and organisms removed, and added to the material from the sampler. This debris with the organisms was preserved in 10% buffered formalin. Where the volume of debris was large, samples were stained with rose bengal prior to examination. The invertebrates were identified to family and species when possible, counted, and the biomass of each family weighed.

The ability of the fauna to reestablish their populations after the treatment was examined at one treated and one untreated riffle in Achigan Creek. Six replicate samples were collected inside an area of 1.8 m^2, marked off at each site 6 h prior to TFM treatment. These areas were then dug up in order to establish a near absence of the bottom fauna (Waters 1965), and the gravel was retramped. When resampled 3 wk later, four samples each were collected from inside (near the centre) and outside of the disturbed areas at both sites.

Daytime drift samples were collected 3 d before, during, and 4 d after the lampricide treatment of Soper Creek. Sets of approximately 2-h duration were made between 09:30 and 17:30. Four 30 × 45 cm drift nets (500 and 360 μm) were placed in 27–32 cm of water below a swift riffle. Flow rates were

measured inside and outside the net mouths with an electronic flow meter to calculate the water filtered by each net, as well as the total stream flow at the site. Mean abundances of drifting animals per 1000 m^3 were calculated prior to analysis, and can be converted to numbers per square metre per hour by multiplying by the average current speed.

Results

Softwater Community

Invertebrate populations in the softwater Goulais River were naturally low (Table 2), while numbers were slightly higher in smaller Achigan Creek. In both streams the substrate was smooth, unstable cobble, with little periphyton. The stream characteristics at the sample sites are listed in Table 1. The number of taxa in the treated section of the Goulais dropped from 23.3 to 20.0 over the sample interval, while in Achigan Creek the number decreased from 29 to 26 taxa.

Three weeks after the lampricide treatment, the philopotamid caddisflies (*Dolophilodes* sp. and *Chimarra* sp.) were absent at all treated riffles in the Goulais–Achigan Creek system (Table 2), while there was little change in their abundance at the untreated riffles (ANOVA; $p < 0.05$). Oligochaete populations in the Goulais River were insufficient for analysis; however, at the treated riffles in Achigan Creek, the abundance of Lumbriculidae (*Stylodrilus*) after treatment was significantly reduced by 70% ($p < 0.05$). The chloroperlid stoneflies were reduced ($p < 0.05$) at only the treated sites in the Goulais River. The responses of the Tipulidae and Chironomidae to the treatment were less clear, as they were significantly reduced at one site, increased significantly at another, while no change occurred at the other treated sites.

The Baetidae (*Pseudocloeon parvulum*) were reduced at only one treated riffle in the Goulais River; however, the siphlonurid (*Isonychia bicolor*) population dropped at the control area. The other riffle mayflies, Heptageniidae (primarily *Heptagenia flavescens* grp. and *Rhithrogena* sp.) and the Leptophlebiidae (*Paraleptophlebia mollis*) increased significantly (ANOVA; $p < 0.01$) at five of the six riffles sites over the 3-wk post-treatment period (Table 2). The nymphs must have arrived as drift, as their size ruled out a new generation.

The proportion of Trichoptera in the community biomass underwent a major drop (from 20 to 8%) in the treated sections of the Goulais River system, whereas their proportion in the control sections increased. The greatest decrease (from 204 to 46 $mg \cdot m^{-2}$) occurred at the treated section (stn. 2) of Achigan Creek. Likewise, oligochaete biomass dropped from 31 to 6 $mg \cdot m^{-2}$ in the treated section. The only other order displaying a large reduction in percent biomass following treatment was the Plecoptera, which dropped from 37 to 12% in the Goulais River (175–62 $mg \cdot m^{-2}$). However, this drop was not evident in Achigan Creek.

With the exception of the Philopotamidae, the dominant invertebrates were able to recolonize the disturbed areas in Achigan Creek over the 3 wk following the lampricide treatment. At the treated riffle, ANOVA displayed no differences between the abundances of each family present on the disturbed area versus the undisturbed area of the streambed. Only the Heptageniidae at the untreated control riffle (stn. 3) were less numerous inside than outside the disturbed area (ANOVA; $p < 0.05$; df = 1, 6).

Hardwater Community

In Soper Creek the invertebrate populations were much more numerous than in the more northern Goulais River system, averaging 2907 $\cdot m^{-2}$ (SE = 361) and 2619 $\cdot m^{-2}$ (SE = 258) before and after treatment. With more consistent flow, the substrate was more stable, while periphyton was greater than that in the Goulais River. The number of taxa was constant at the upper riffle (19) but increased from 15 to 18 at the lower riffle 4 d after the treatment. Species occurrence at the two dissimilar riffles in Soper Creek was similar (Table 3), with the percent composition of the Ephemeroptera and Chironomidae being the same. However, before the treatment the Trichoptera were twice as important at the upper riffle (stn. 1) on cobble than at the lower riffle. Annelida accounted for 20% of the fauna at the lower riffle (stn. 2) on poorly sorted gravel and sand, as compared with only 4% at the upper.

The Annelida were reduced in Soper Creek following the lampricide treatment. Initially, the oligochates (mainly *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*) comprised 53% of the total dry biomass at the lower riffle. This dropped to 12% 4 d after treatment. All the annelid families present decreased similarily after treatment. The leeches (Piscicolidae) were most affected, with a drop in biomass from 61 to 10 $mg \cdot m^{-2}$. Decaying leeches were present at an average of 76 $\cdot m^{-2}$ in the post-treatment Surber samples, in addition to those presumed alive at the time of sampling. Turbellaria were also reduced significantly (ANOVA; $p < 0.01$), while less significant reductions occurred in *Asellus intermedius*, *Syndiamesia* sp., and the Nemouridae.

The community composition at the lower riffle changed following the treatment, where the percent biomass of the Ephemeroptera and Trichoptera increased from 10 to 56% and 3 to 8%, respectively. Numbers of several species (*Simulium vittatum*, *Hydropsyche* spp., and *Baetis cinqulatus*) increased significantly ($p < 0.01$) at the lower riffle immediately following treatment (Table 3). At the upper riffle, only the Simuliidae and Elmidae increased in abundance ($p < 0.1$) following the lampricide treatment. The biomass of most families remained similar at the upper riffle following the treatment. However, biomass of the Trichoptera and Annelida dropped from 432 to 277 $mg \cdot m^{-2}$ and 52 to 5 $mg \cdot m^{-2}$, respectively.

Induced Drift

Daytime drift rates indicated a catastrophic drift of leeches during the treatment (Table 4), as their biomass (dry weight) in the drift increased from 0.005 to 2.61 $g \cdot 1000\ m^{-3}$. Except for the annelids, hydrophilid Coleoptera, and emerging Chironomidae, the proportion of each family in the drift 3 d before treatment was similar to that 4 d after the treatment. All oligochaete families drifted more ($p < 0.01$) during the lampricide treatment than before or after the treatment (Table 4). Other genera showing increased drift only during treatment were *Glossosoma*, *Antocha*, *Syndiamesia*, and *Gammarus* ($p <$ 0.05–0.1), as well as the Turbellaria (including gill flukes *Octomacrum*).

The chemical first reached the drift sampling site after 05:00, May 6. Stressed lamprey ammocoetes were first noticed after 09:45, while none were collected in the drift nets after 12:00. Likewise, the numbers of drifting Ephemeroptera and Trichoptera dropped progressively during the treatment, indicating greatest impact during the first 6 h of treatment. This was

TABLE 2. Mean numbers of invertebrates (m^{-2}) at treated and control riffles in
June 1982. Only families present at two or more riffles are listed. Probabilities (p)
df = 1, 9 for Goulais; df = 1, 12 for Achigan.

	Goulais						
	Treated						
	Stn. A			Stn. B			Co
	Pre	Post	p	Pre	Post	p	Pre
Lumbriculidae	1.83	0		2.2	0		—
Tubificidae	—	—		—	—		1.9
Ephemerellidae	3.7	2.2		1.8	0		1.8
Baetidae	20.2	3.7	*	0	4.4		—
Leptophlebiidae	16.6	17.6		0	13.0	**	1.8
Heptageniidae	137.5	367.2	*	46.1	126.1	***	35.0
Siphlonuridae	11.1	3.7		12.9	4.4		20.3
Hydropsychidae	50.0	68.7		20.3	31.0		48.1
Glossosomatidae	1.8	0		—	—		1.8
Rhyacophilidae	—	—		—	—		—
Philopotamidae	70.1	0	***	14.8	0	**	35.1
Limnephilidae	—	—		1.8	0		—
Polycentropidae	0	4.4		1.8	2.2		—
Leptoceridae	—	—		—	—		0
Pteronarcidae	1.8	0		—	—		—
Perlidae	3.7	8.9		5.5	4.4		7.3
Chloroperlidae	7.3	0	*	1.8	0		—
Leuctridae	—	—		—	—		—
Gomophidae	7.3	11.0		0	4.4		16.5
Aeshnidae	—	—		—	—		1.9
Elmidae	—	—		0	2.2		—
Curculionidae	0	11.0		1.8	5.5		0
Tipulidae	1.8	4.4		11.0	44.3	*	0
Simuliidae	0	2.2		22.2	5.6		—
Athericidae	—	—		—	—		0
Empididae	—	—		0	2.2		—
Ceratopogonidae	—	—		—	—		—
Tanypodinae	1.8	4.4		1.8	0		1.9
Orthocladiinae	22.0	8.9		18.4	6.5		3.7
Chironomini	88.6	28.5	**	77.5	51.0		27.9
Total no.·m^{-2}	449.0	546.7		239.6	307.8		206.9
Total biomass (g dry wt·m^{-2})	0.66	0.58		0.17	0.26		0.4

riffles in the Goulais River and Achigan Creek, sampled 1 d before and 3 wk after TFM treatment, lities (p) are from ANOVA values after $X^{0.25}$ transformation: *$p < 0.01$; **$p < 0.05$; ***$p < 0.01$;

			Achigan								
			Treated								
Control, stn. X			Stn. 1			Stn. 2			Control, stn. 3		
Pre	Post	p	Pre	Post	p	Pre	Post	p	Pre	Post	p
—	—		168.4	62.2	**	31.5	6.9	**	0	1.3	
1.9	0		—	—		—	—		1.9	0	
1.8	0		3.7	6.6		9.2	5.5		—	—	
—	—		3.7	4.4		0	8.3		0	4.2	
1.8	2.2		18.5	57.7	**	27.7	149.7	***	5.5	130.4	***
35.0	50.9		88.6	99.9		105.4	255.3	***	83.2	135.8	*
20.3	0	*	—	—		—	—		—	—	
48.1	33.2		31.4	17.7		93.9	48.4		33.4	74.9	**
1.8	0		7.4	0		3.7	0		7.4	0	
—	—		0	2.2		0	1.4		—	—	
35.1	15.5		20.3	0	**	81.4	0	***	20.4	69.4	
—	—		1.9	0		—	—		—	—	
—	—		7.4	2.2		—	—		0	2.8	
0	4.4		1.9	0		—	—		—	—	
—	—		—	—		0	1.4		0	1.4	
7.3	0		14.7	22.9		11.1	24.9		3.7	8.3	
—	—		—	—		1.9	1.4		0	2.8	
—	—		0	2.2		0	2.8		0	6.9	
16.5	15.5		9.2	13.3		5.5	11.0		3.7	2.8	
1.9	0		1.9	2.2		0	1.4		—	—	
—	—		7.4	17.7		1.9	5.5		0	4.2	
0	4.4		—	—		—	—		0	2.8	
0	2.2		7.4	0	*	5.5	2.8		0	5.5	
—	—		0	4.4		9.2	4.1		1.8	6.9	
0	2.2		12.9	8.7		16.6	18.1		1.9	2.8	
—	—		0	2.2		—	—		0	1.4	
—	—		5.5	2.2		1.9	1.4		—	—	
1.9	0		0	11.1		1.8	1.4		3.7	2.8	
3.7	4.4		195.9	46.5	**	11.3	26.0		9.3	20.8	
27.9	6.6		72.1	53.2		24.0	47.2	*	26.2	22.2	
206.9	141.6		681.9	443.9		447.1	625.4		207.4	511.7	
0.49	0.17		0.43	0.54		0.41	0.77		0.16	0.37	

TABLE 3. Mean numbers ($0.1 \cdot m^{-2}$) and total dry biomass ($g \cdot m^{-2}$) of invertebrates from riffles in Soper Creek sampled 3 d before and 4 d after lampridcide treatment on May 6, 1983. Probabilities are from ANOVA values after $X^{0.25}$ transformation: ns, not significant; $*p < 0.1$; $**p < 0.05$; $***p < 0.01$; df = 1, 20.

	Stn. 1			Stn. 2		
	Pre	Post	p	Pre	Post	p
Baetidae	73.0	98.0		3.9	47.0	***
Ephemerellidae	1.3	1.6		0.6	0.2	
Leptophlebiidae	2.0	5.0	***	0.7	2.3	***
Heptageniidae	1.4	1.4		0	0.2	
Hydropsychidae	53.6	32.5		1.4	9.5	***
Rhyacodrilidae	0.9	0.8		0.1	0.1	
Glossosomatidae	0.1	0.2		0	0.1	
Tipulidae	12.4	16.4		4.1	16.5	ns
Simuliidae	2.7	10.3	*	0.9	6.1	***
Ceratopogonidae	20.7	4.6	**	3.6	0.6	
Tanypodinae	0	0.2		0.3	0.1	
Diamesinae	1.8	0.4	***	1.3	2.4	
Orthocladiinae	90.1	64.6	ns	13.3	41.3	ns
Chironomini	4.0	4.8		0	4.0	ns
Tanytarsini	2.5	3.5		2.3	14.0	ns
Pupa and adults	14.3	22.7	ns	1.9	13.0	ns
Neumoridae	0.4	0.1		0	0	
Elmidae	1.7	2.9	**	0.4	1.6	ns
Gammaridae	0.2	0		0	0.1	
Asellidae	0.3	0.2		0.4	0.1	*
Lumbriculidae	6.0	5.7		62.6	20.5	**
Tubificidae	1.1	0.7		9.4	8.3	
Naididae	0.3	0.1		0.6	0.1	*
Eiseniella	—	—		0.3	0.1	
Piscicolidae	5.8	0.9	***	10.4	2.1	***
Nematoda	2.3	3.7		3.0	2.5	
Turbellaria	0.2	0	*	0.3	0	**
Total abundance (no. $\cdot 0.1\ m^{-2}$)	298.4	283.1		121.1	195.5	
Total biomass (g dry wt $\cdot m^{-2}$)	1.52	1.53		0.46	0.79	

TABLE 4. Mean numbers ($1000 \cdot m^{-3}$) and total biomass ($g \cdot 1000\ m^{-3}$) of daytime drift in Soper Creek sampled before, during, and after lampricide treatment. Probabilities (p) are from ANOVA values after $X^{0.25}$ transformation: ns, not significant; $*p < 0.1$; $**p < 0.05$; $***p < 0.01$; df = 2, 28. Only probabilities without pre vs post differences are listed.

	Pre, May 3	During, May 6	Post, May 10	p
Baetidae	21.66	72.73	76.02	ns
Leptophlebiidae	0.19	1.26	1.01	
Heptageniidae	0.24	0.46	0	
Hydropsychidae	3.9	7.8	4.7	
Rhyacodrilidae	0.2	0.3	0	
Glossosomatidae	0	0.3	0	*
Limnephilidae	0	0.2	0	
Tipulidae	2.2	7.9	5.4	**
Simuliidae	1.6	22.0	27.9	ns
Ceratopogonidae	5.5	14.7	4.5	
Empididae	0	0.6	0	
Tanypodinae	2.0	0.2	1.8	
Diamesinae	0.7	5.8	0	*
Orthocladiinae	87.9	166.4	110.6	***
Chironomini	0	4.5	0	
Tanytarsini	1.6	3.7	1.8	
pupa and adults	36.7	241.9	491.6	ns
Elmidae	0.4	0.8	0.3	
Hydrophilidae	0	0.6	2.8	ns
Haliplidae	0.2	0.2	0.5	
Collembola	0	6.7	18.1	
Neumoridae	0.2	0	0	
Gammaridae	0.4	4.1	0.4	**
Asellidae	0.2	2.4	2.8	ns
Lumbriculidae	2.6	16.6	0.5	***
Tubificidae	2.2	7.9	5.4	***
Naididae	2.4	12.6	0	***
Eiseniella	0	1.0	0.5	
Piscicolidae	7.5	3642.9	5.7	***
Erpobdellidae	0	0.9	0	**
Nematoda	1.7	5.2	0	
Turbellaria	0	6.0	0	***
Total abundance	182.8	4258.2	762.9	
Total biomass (g dry wt $\cdot 1000\ m^{-3}$)	0.05	2.76	0.15	

opposite the trend for the drifting mayflies on May 10, numbers of which increased as water temperature rose with time (Fig. 1). Spring emergence was occurring during the sampling, with *Baetis cingulatus* and *Crictopus* sp. displaying a progressive increase in drift over the sample period, apparently associated with emergence rather than treatment. During the late afternoon of May 10, 50% of the mayfly and 80% of the total chironomid drift was composed of adults as compared with 20 and 15%, respectively, on May 3. Activity of the Simuliidae larvae and Collembola also increased over the sample period, with greatest drift occurring 4 d after the lampricide treatment.

Discussion

The treatment of Soper Creek with TFM + 0.8% Bayer 73 (TFM-2B) caused an obvious mortality of the Turbellaria and Annelida, especially the Piscicolidae. Gilderhus and Johnson (1980) noted that this combination of lampricide is more lethal to some invertebrates than TFM alone. In their review, the 24-h LC50's of the combination were $1.5\ mg \cdot L^{-1}$ for Turbellaria, 2.2 for *Tubifex*, and 4.0 for the Erpobdellidae, at an alkalinity of 130 meq. As the 1983 treatment of Soper Creek was at least $6.3\ mg \cdot L^{-1}$, these organisms would be expected to suffer significant mortality. Likewise, several insects (*Chimarra*, *Simulium*, and *Baetis*) are known to be sensitive to TFM at the levels used. Partly due to their size, there have been few observed mortalities of invertebrates during treatments. A major exception is the large burrowing mayfly *Hexagenia* (Gilderhus and Johnson 1980).

All the oligochaete families present were significantly affected by the lampricide treatments. Maki and Johnson (1977) found a rapid uptake, but very long elimination time, of ^{14}C-labelled TFM in unspecified annelid worms. Scott et al. (1984) found a large reduction of pond oligochaetes, particularly the delicate Naididae, following TFM treatment of experimental ponds. Subsequent data also revealed a large increase of drifting

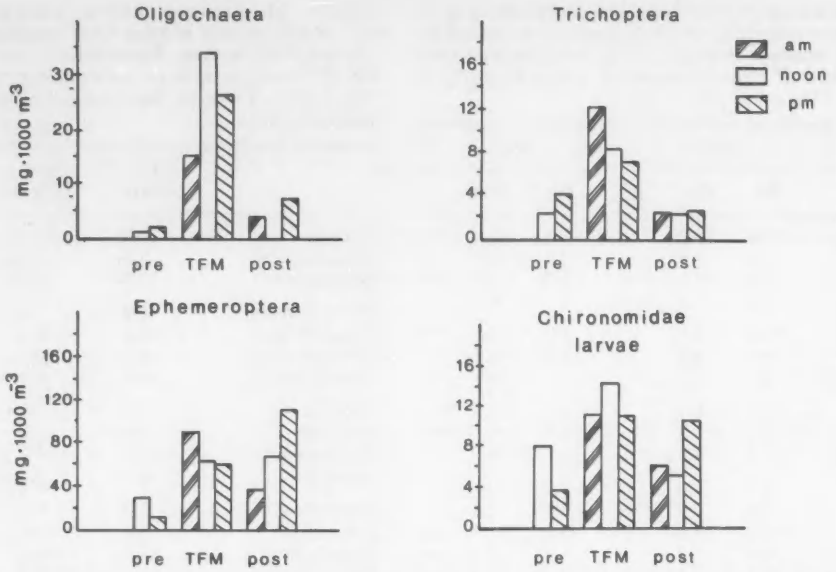

FIG. 1. Mean biomass of daytime drift in Soper Creek before, during, and after the 1983 lampricide treatment.

naidids during the lampricide treatment of a stream neighbouring Soper Creek. Naidids are capable of active swimming, in contrast with the Lumbriculidae and Tubificidae (Brinkhurst and Jamieson 1971), whose presence in drift samples is primarily through passive transport (Larimore 1974). Their avoidance activity obviously increased during the treatment, resulting in significantly greater drift. Had the worms been killed in the substrate, they would remain there and not reenter the drift (Walton 1978).

Wallace et al. (1973) found that catastrophic drift of non-target invertebrates occurred in Quebec streams in association with the use of black-fly larvicides, although Surber samples failed to reveal extensive mortalities in the streams. Whereas the arthropods *Glossosoma*, *Antocha*, and *Gammarus* all had significant increased drift during the TFM treatment, only *Syndiamesia* sp. displayed a parallel reduction in abundance at the riffle sites. This indicates that increased drift and not invertebrate mortality is the major impact of the lampricide treatments on the riffle communities.

Hynes and Harper (1972) found limited drift of *G. pseudolimnaeus* in the nearby Credit River, where drift averaged $2 \cdot 1000\,m^{-3}$ over 24 h. As the majority of *Gammarus* drift at night (Waters 1965), the increased daytime drift in Soper Creek is more significant. During the treatment, 65% of the drifting *G. pseudolimnaeus* were newly hatched young (2 mm) collected in nets set between 12:00 and 14:00. These young *Gammarus* may have been aborted by the ovigerous females, which would be carrying young in May (Hynes and Harper 1972).

The increase in abundance of several insects at the lower riffle in Soper Creek following the treatment is attributed to reattachment of the drifting larvae. Reattachment of black-fly larvae was found on several occasions by Wallace et al. (1973) following stream treatment with insecticides. Hall et al. (1980) noted that pools serve as catch basins for stream drift. The lower riffle was immediately below a large pool where a portion of the drifting *Hydropsyche*, *Simulium*, and mayflies settled, on marginal habitat for these genera. Downstream of this area, the habitat drastically changes as the stream meanders across the sandy, glacial plain to present Lake Ontario (Weber and Morwick 1946). During the lampricide treatment, any *Heptagenidae*, *Hydropsyche* spp., and *Simulium vittatum* drifting beyond this area were removed from the stream community, being unable to maintain themselves on sand (Luedtke and Brusven 1976).

Seasonal cycles in temperate streams hinder population comparisons, even over a 3-wk interval, considering the emergence cycles and changes in microhabitat that can occur in rivers with low summer flows (DeMarch 1976). Separation of temporal changes from the impact of lampricides on the fauna requires that populations change only in the treated sections of the streams. The only insects whose elimination can be positively associated with the TFM treatment of the Goulais system were the Philopotamidae (*Dolophilodes* and *Chimarra*), with no reduction occurring in the untreated portion of the two streams.

Merna (1982), in his comparison of lampricide treated and nearby untreated sections of three Michigan trout streams, all with consistent application points, found several insect species to be less abundant in the treated areas 1–2 yr after lampricide treatment. Merna interpreted the differences to the long-term impacts of the repeated treatments. This would apply to the biennial *Hexagenia* but may not apply to those species with shorter generation times. Many of the affected species, particularly the *Ephemerella* spp. and *Drunella* spp., were not present in this study, while the genera *Glossosoma*, *Paraleptophlebia*, and *Rithrogena* displayed no reduction in either Soper Creek or the Goulais system. Precise literature comparisons are difficult due to variable species sensitivity and treatment concentrations. However, comparisons between this study and the surveys of Torblaa (1968) and Merna (1982) imply consistent declines in the Nemouridae, Philopotamidae, and oligochaetes in stream sections treated with lampricides.

Downstream drift helps to reestablish and redistribute many invertebrates in streams that are denuded by natural or pollution events (Waters 1965; Williams 1977). Streams are normally treated with TFM from a point above which ammocoetes are rare. Thus, untreated areas exist upstream to act as sources for recolonization, should the fauna suffer adverse mortality. Shaw and Minshall (1980) found that colonization rates varied by taxon, with most peaking within 64 d. However, the Philopotamidae did not reappear at any of the treated riffles, in spite of continuing populations upstream. Three weeks was too short an interval to enable this caddisfly to migrate the 6-km distance from above the application point. Oligochaetes were rare in the untreated section, so upstream movement (Williams 1977) or vertical movement from refugia deep in the substrate would be prevalent in reestablishing their populations. Torblaa (1968) found that the riffle fauna in several Michigan streams recovered within 6 wk and had returned to pretreatment abundance within a year following lampricide treatment. Over the 3-wk interval of our study, most invertebrates were able to recolonize the physically disturbed areas of streambed. However, the oligochaetes and Philopotamidae did not recover to their pretreatment abundance.

In general, limited direct mortality of the riffle fauna was found. Few of the prominant insects were adversely affected by the treatments, while recolonization was capable of reestablishing most populations within a month. Upstream, untreated refugia exist from which most univoltine and multivoltine species can repopulate the treated areas within a summer through reproduction and downstream drift. An exception are the more sensitive pool fauna, adapted to lower oxygenated environments. Having either thin cuticles or large gill surfaces, they are known to be susceptible to the levels of TFM normally used (Maki and Johnson 1977; Gilderhus and Johnson 1980). Annelids appear most affected, as recolonization is mainly from survivors deep in the substrate. Although minor components of the fauna in gravelly streams, they are important in sandy areas, together with the sensitive mayfly *Hexagenia* and sphaerid clams. These organisms have long generation times of up to 2 yr, limiting their recovery prior to the succeeding treatment.

Given the high rebound capacity of stream invertebrates following periodic drought and flood events, the effects of treatments at 3- to 4-yr intervals would not be expected to last more than one generation. The effects on growth rates and productivity in streams require long-term studies, as treatments alter species composition and could change the age composition of a sensitive population (Resh 1982). As natural drift is considered production in excess of the carrying capacity of the stream (Waters and Hokenstrom 1980), the copious drift during lampricide treatment temporarily reduces the potential production of the stream. This study cannot answer if the populations remaining quickly fill the carrying capacity of the stream, resulting in no net loss of productivity.

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Phosphorus Flux through Epilimnetic Zooplankton from Lake Ontario: Relationship with Body Size and Significance to Phytoplankton

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Taylor, W. D. 1984. Phosphorus flux through epilimnetic zooplankton from Lake Ontario: relationship with body size and significance to phytoplankton. Can. J. Fish. Aquat. Sci. 41: 1702–1712.

The flux of phosphate through epilimnetic zooplankton from Lake Ontario was measured by adding $^{32}PO_4$ into whole lakewater, monitoring its accumulation by zooplankton over 48 h, and then measuring its release when animals were returned to unlabelled lake water. Phosphorus uptake was calculated as the sum of accumulation and release rates. The 10 species examined ranged in size from 36-μm *Codonella cratera* to 1-mm *Daphnia retrocurva*. Phosphorus uptake rates of herbivorous zooplankton ranged about 200-fold and increased with body size both within and among species, although not proportionally. Among species, there was a pronounced allometry, with smaller species having much higher rates per unit size. This allometry is similar to that described for other metabolic parameters and body size. There was no tendency for smaller species to release a greater fraction of the label they took up. These results suggest that the biomass of zooplankton consuming a given phytoplankton production will be much smaller if small zooplankton dominate, and therefore, this phosphorus sink will be much smaller. Further, the flow of phosphorus to higher trophic levels may be reduced. The result of changing the zooplankton size-distribution may be to change the amount of spring total phosphorus available to summer phytoplankton.

Pour mesurer le flux de phosphate dans le zooplancton épilimnétique du lac Ontario, nous avons mélangé du $^{32}PO_4$ à l'eau du lac, puis nous en avons surveillé la concentration dans le zooplancton pendant une période de 48 h ; ensuite, après avoir placé les organismes dans de l'eau non marquée, nous en avons mesuré la libération. Nous avons déterminé l'absorption de phosphore en additionnant les taux de concentration et de libération. La taille des 10 espèces différentes que nous avons étudiées variait de 36 μm (*Codonella cratera*) à 1 mm (*Daphnia retrocurva*). Le taux d'absorption chez les organismes herbivores du zooplancton variait par un facteur de 200, augmentant, quoique non proportionnellement, en fonction de la taille de l'organisme et ce, chez une même espèce ainsi que d'une espèce à l'autre. L'allométrie entre les espèces était marquée ; le taux par unité de taille était beaucoup plus élevé chez les espèces de petite taille. L'allométrie observée ici est semblable à celle qu'on a remarquée pour d'autres paramètres du métabolisme étudiés en fonction de la taille de l'organisme. Les espèces de petite taille ne manifestaient aucune tendance à libérer une plus grande proportion de marqueur. Ces résultats indiquent que la biomasse du zooplancton consommant une quantité donnée de phytoplancton sera beaucoup plus petite si les organismes de petites taille dominent dans le zooplancton et, dès lors, que l'effet de rétention du phosphore sera beaucoup moindre. En outre, le flux de phosphore vers les niveaux trophiques supérieurs pouvait être réduit. La modification de la répartition des tailles des organismes du zooplancton pourrait avoir pour effet de changer la quantité totale de phosphore retenu au printemps devenant assimilable en été pour le phytoplancton.

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Investigations of the eutrophication process in lakes have mainly involved the relationship between the absolute and relative amounts of potentially limiting nutrients and the mass and species composition of phytoplankton. A smaller but expanding literature deals with effects generated by higher trophic levels. Particularly interesting with respect to lake management are a large number of papers documenting an increase in phytoplankton biomass and a shift toward net phytoplankton coincident with a decrease in large herbivorous zooplankton (Hrbáček et al. 1961; Hurlbert et al. 1972; Andersson et al. 1978; Stenson et al. 1978; Leah et al. 1980; Henrikson et al. 1980; Lynch and Shapiro 1981; Elliott et al. 1983; Vyhnálek 1983). A change in the stock of large zooplankton is readily effected by changing the fish community (Hrbáček et al. 1961; Brooks 1968; Wells 1970; Henrikson et al. 1980; Stenson 1982), a common result of human activity. It is now clear that an adequate understanding of eutrophication must be a holistic one which includes all trophic levels.

The objective of this work was to gain some insight into why a shift in zooplankton species composition from large to small might affect phytoplankton. For example, one mechanism that has been proposed is that small zooplankton are more active in nutrient regeneration (Nilssen 1978; Lehman 1980; Bartell 1981; Stenson 1982), although early measurements of high phosphorus release by ciliates have recently been questioned (Taylor and Lean 1981). I examined phosphorus flux through

TABLE 1. Physical and chemical conditions during the five cruises. All data are for the 5-m depth. Concentrations are in $\mu g \cdot L^{-1}$. Turnover time is in minutes.

Station and date of experiment	Temperature (°C)	Chlorophyll	Particulate organic carbon	Total phosphorus	Soluble reactive phosphorus	Particulate carbon/ phosphorus	Phosphate turnover time
1. Station 401 (43°53′29″N, 78°16′27″W), June 29 – July 3, 1981	13.0	—	673	17.0	<0.2	56.1	13
2. Station 401, June 7–11, 1982	9.5	2.9	492	19.7	0.6	35.6	43
3. Station 401, June 28 – July 2, 1982	8.6	5.8	675	13.4	2.0	160.7	49
4. Station 403 (43°16′12″N, 78°13′58″W), Aug. 23–27	13.4	3.4	408	13.6	<0.2	43.9	8.5
5. Station 404 (43°26′39″N, 78°12′12″W), Sept. 20–24	15.6	4.7	489	14.9	1.1	67.2	—

the most abundant epilimnetic grazers in Lake Ontario, covering as wide a range in body size as possible, from *Codonella cratera* (36 μm) to *Daphnia retrocurva* (1 mm). These results are examined with respect to allometry in phosphorus flux, the potential contribution of zooplankton to total nutrient flux through the phytoplankton, and the possible significance to phytoplankton of shifts in zooplankton size-distribution. Note that throughout, "phytoplankton" will refer to all heterotrophic and autotrophic microorganisms using inorganic phosphorus.

Materials and Methods

I measured nutrient uptake by zooplankton by adding carrier-free $^{32}PO_4$ to whole lake water, following its movement into phytoplankton by filtering at various times to collect particulate ^{32}P, and then following its movement into zooplankton by collecting individuals over 48 h and determining their ^{32}P content. This rate of labelling, or ^{32}P accumulation rate, requires that the phytoplankton be labelled relatively quickly. It is an underestimate of assimilation rate to the extent that assimilated phosphorus compounds are respired, and could be considered an estimate of growth rate. After about 48 h, the rate of ^{32}P release is measured by placing the animals in unlabelled lake water. The sum of accumulation and release rates is taken as uptake. The rationale for these measurements is illustrated in Fig. 1. Notice that uncertainties about the path of ^{32}P through the zooplankton do not affect the measure of these fluxes.

I have translated rates of ^{32}P flux measured in disintegrations per minute (DPM) to phosphorus by dividing DPM by the activity of the labelled lake water (DPM divided by DPM per microlitre) to express phosphorus as microlitres of total phosphorus. This is convenient for comparison between times and sites and for comparing zooplankton fluxes to orthophosphate flux measured by $^{32}PO_4$ uptake. Weight of phosphorus released can be obtained by multiplying by the total phosphorus concentration (Table 1).

Experiments were carried out in Lake Ontario at three stations (Table 1). Station 401 is about 4 km south of Cobourg, Ontario, at a depth of 40 m, and is stratified in June, while offshore stations are not. Station 403 is near the centre of the lake at a depth of 178 m. Station 404 is just south of 403 and was used

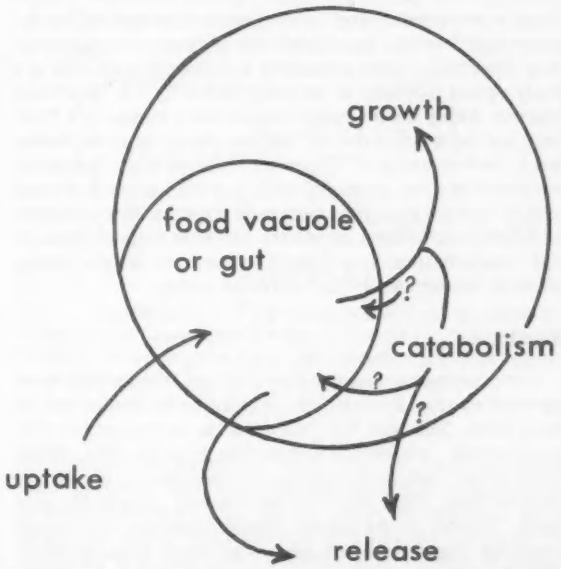

FIG. 1. Schematic diagram of a zooplankter, showing the fluxes measured. Notice that many uncertainties concerning the components and release are not important to the measurements.

rather than 403 in September 1982 because it was the last station visited by the research ship *Limnos* during the cruise. Water was collected, spiked (see below), and brought to the University of Waterloo, where experiments were carried out. All other work was done on the *Limnos* with incubations in situ.

Experimental procedure is similar to Taylor and Lean (1981). Water was collected from the 5-m depth, placed in 2-L polyethylene bottles, spiked with about 5.0 GBq of carrier-free $^{32}PO_4$, and incubated. Smaller quantities of water were spiked also; a weaker spike was used to monitor $^{32}PO_4$ uptake by the plankton over the first 1–2 h and to determine orthophosphate turnover time. A stronger spike was sometimes incubated for ^{32}P release experiments on small animals, such as ciliates and rotifers, and incubated in situ also.

The uptake of $^{32}PO_4$ by the plankton was determined by filtering 1-mL aliquots of water through 0.2-μm Nuclepore polycarbonate filters. Distribution of ^{32}P among plankton size-classes was determined by filtering 1- to 5-mL aliquots through 0.2-, 1.0-, 5.0-, and 12-μm Nuclepore filters. Filters were placed in scintillation vials, dissolved, and assayed for radioactivity by scintillation counting.

Removal of zooplankton from the labelled lake water began after 1–3 h of incubation and continued until 30–48 h. Aliquots of labelled water were decanted into petri dishes, and individual zooplankters were removed with finely drawn pasteur pipets. They were transferred through three 0.5- to 1.0-mL washes in filtered (15 μm), unlabelled lake water and placed in a scintillation vial. Crustaceans were measured using an ocular micrometer at 40× magnification before adding fluor (PCS, Amersham). This procedure takes about 30 s, and transfers a negligible amount (1×10^{-5} μL) of labelled lake water to the vial along with the animal.

Phosphorus release experiments began after 48 h of incubation. Single animals were quickly washed as above and placed in a well (Pyrex 3-spot depression plate) containing 1 mL of filtered (15–150 μm, depending on species) lake water. Coarse filtration prevented unlabelled zooplankton from getting into the experimental vessel, but allowed the presence of food organisms. Depression plates containing zooplankton were kept in a dimly lighted incubator at lake temperature for 1 h. At 10-min intervals during the hour, the animals were moved to a fresh well, and the water in the old well was placed in a scintillation vial for determination of ^{32}P content. After the hour, the animal was placed in a vial, measured in the case of crustacea and some rotifers, and put aside for scintillation counting. Size estimates for ciliates and rotifers are means based on measurements at 400× magnification from Lugol's iodine fixed samples during plankton enumeration by the Utermohl method.

Results

June experiments were conducted inshore (station 401) when the water was cool but stratified, being inside the thermal bar. At these times, SRP was low, but demand, as indicated by the turnover time, was only moderate. The June 28, 1982, cruise followed an inshore upwelling, as reflected in the longer turnover time. Once the offshore stratified, station 401 was highly variable in phosphorus demand because of frequent upwelling. August and September experiments were conducted offshore; surface waters were generally warmer, and phosphorus demand, as indicated by the turnover time, was greater.

On adding $^{32}PO_4$ to lake water, the label is quickly taken up by the particulate phase, primarily 0.2- to 1.0-μm particles. A significant amount of label is also found in 1.0- to 12.0-μm particles, while relatively less is found in the 0.2-μm filtrate or particles retained by the 12-μm filter (Fig. 2). The size-distribution of label drifted at times (e.g. June 28 cruise) but, in general, the distribution was set up quickly compared with the 48-h incubation and remained relatively constant.

The distribution of chemically determined phosphorus is also skewed towards smaller cells (Table 2). The most striking difference between the distribution of phosphorus (^{31}P) and ^{32}P is in the greater than 12.0-μm size-class. Relatively less of the ^{32}P label is found there, probably reflecting the importance of large, slowly labelled, phagotrophic organisms in this size-class and the inefficiency with which these animals are collected by small-volume pipets for filtration of ^{32}P-labelled water.

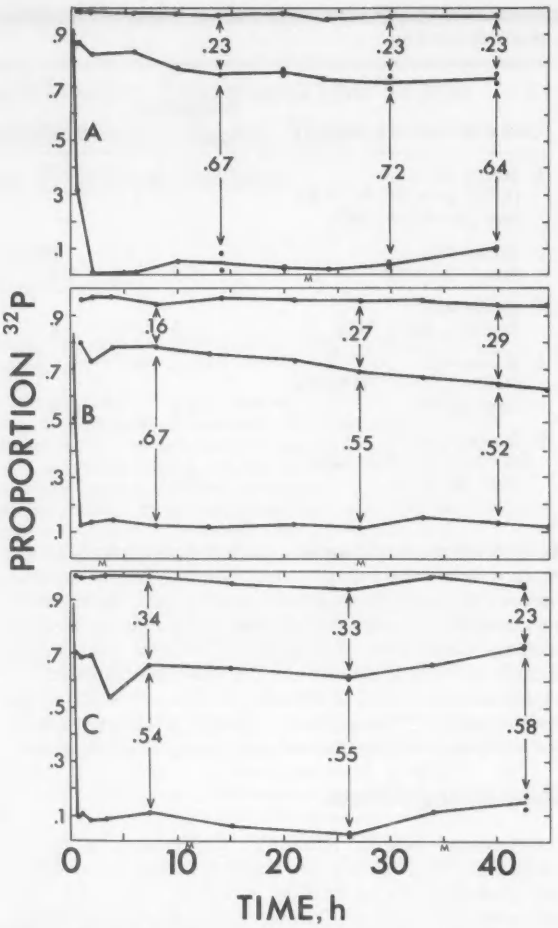

FIG. 2. Proportion of the total ^{32}P passing 0.2-, 1.0-, and 12-μm filters (bottom, middle, and top lines, respectively) during the experimental incubations. (A) Cruise 2; (B) cruise 3; (C) cruise 4. The proportion of ^{32}P, by difference, between 0.2 and 1.0 μm, and between 1.0 and 12.0 μm, is indicated on the graphs. M = midnight (E.D.T.).

The relative proportion of particles from 0.2–1.0 to 1.0–12.0 μm also differs between ^{31}P and ^{32}P. For example, the relative proportion of particles from 1.0–12.0 to 0.2–1.0 μm on June 8 at station 401 was 0.34 for ^{32}P, but 0.56 for ^{31}P (Table 2). On June 28 at station 401 these values were 0.41 compared with 0.63, while on August 22 at station 403 they were 0.55 compared with 1.10, reflecting a maximum twofold difference in specific activity between size-classes.

Phosphorus Accumulation

In contrast with the rapid uptake of label by whole lake water, the labelling of the ciliate *Strombidium viride* is gradual (Fig. 3) and only begins to slow near the end of the incubations. This decline in labelling rate probably results from cell division. Only the first 30 h were used in the calculation of accumulation rates. Although the June 28 data are particularly variable, it appears that accumulation rates are similar for June 7 and June 28 at station 401 and about twice the August 23 rate from station

TABLE 2. Proportions of chemically determined phosphorus in filtration-generated size-classes for the stations and dates shown in Fig. 2. Inorganic particulate phosphorus is excluded. All data are for the 5-m depth.

Station and date	<0.2 μm	0.2–1.0 μm	1.0–12 μm	>12 μm
Station 401, June 8	0.050	0.477	0.266	0.207
Station 401, June 28	0.035	0.535	0.340	0.090
Station 403, Aug. 22	0.060	0.385	0.420	0.135

403. The lower rate in August may reflect a change in specific activity or abundance of preferred food particles. Work in progress on seasonal changes in size and abundance of Lake Ontario ciliates indicates that *Strombidium* at station 401 in June had a mean volume twice as great as *Strombidium* at station 403 in August, so feeding rate probably had declined.

Accumulation rates for other ciliates, especially *Stokesia*, show a decline in labelling rate and a relatively large amount of variability, probably reflecting cell division (Fig. 4). Perhaps an exclusion of low data points from the rate estimates would produce better estimates, but this would be uncomfortably subjective. I have tried to avoid this difficulty by excluding data points after 40 h for ciliates (*Codonella*, *Strobilidium*, and *Stokesia*), the rationale being that many individuals will have divided after this time. (Animals that divide early in the experiment will produce a smaller error.) Rotifers (*Polyarthra*, *Synchaeta*, and *Keratella*) that do not divide by binary fission show no similar increase in variability during the experiment (Fig. 4), although loss of eggs could be contributing to an underestimation of accumulation.

By using the maximum accumulation of ^{32}P reached by *Strombidium*, *Codonella*, *Strobilidium*, and *Stokesia* as measures of the phosphorus content of an individual about to divide, and dividing these by twice the ^{32}P accumulation rate, we obtain crude estimates of generation time: 15–26, 29, 43, and 25 h, respectively. These should be minimum estimates and will be low if the animals are not completely labelled at the end of the incubation.

The ^{32}P accumulation rate for *Polyarthra vulgaris* in Fig. 4 is lower than for other rotifers and is also the only one measured offshore in late summer. Its accumulation rate appears to accelerate during the incubation, a problem more pronounced in *Diacylops* (discussed below). Alternately, a lower ^{32}P accumulation rate in offshore waters could be a general phenomenon, not restricted to *Strombidium*.

Attempts were made to measure accumulation rates for other species of ciliates. Some (e.g. *Dileptus*) were not abundant enough to collect sufficient data. Two species, *Coleps* sp. and a scuticociliate, which is probably undescribed (D. Lynn, University of Guelph, Guelph, Ont., pers. comm.), did not accumulate measurable amounts of ^{32}P. They could have very low feeding rates, a low phosphorus content, or feed on something not labelled by $^{32}PO_4$ addition. (*Coleps* is a facultative histiophage and could be feeding on dead zooplankton.) However, the cause of their apparent lack of ^{32}P accumulation cannot be judged from these data.

Cyclopoid copepods were very abundant during the June cruises and were almost entirely *Diacyclops bicuspidatus thomasi*. A linear fit to the accumulation of ^{32}P by cyclopoid nauplii (Fig. 5A) had a large intercept; the actual ^{32}P accumulation rate is probably accelerating, and the linear fit probably underestimates the true rate. Nauplii and copepodids apparently

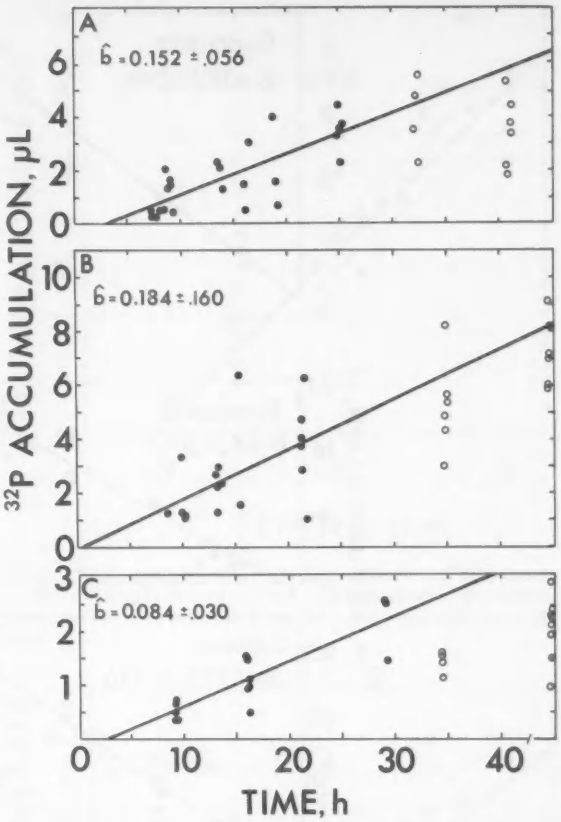

FIG. 3. Accumulation of ^{32}P by *Strombidium viride* at station 401 during the same cruises shown in Fig. 2. Estimates of slopes and 95% confidence limits are given. Open circles represent points not included in the regressions.

have similar accumulation rates. The accelerating rate of labelling results from feeding on items (probably other animals) slowly labelled by $^{32}PO_4$ addition. Rates for individual copepodids and adults were calculated by taking the amount of ^{32}P accumulated by individuals collected after 30–48 h of incubation and dividing by the time less the time-intercept. These rates can be compared with body size (Fig. 5B). Although they are underestimates, they can be compared and indicate that accumulation rate increases with body size.

The relationship between body length and phosphorus accumulation rate for *Bosmina* is approximately linear (Fig. 6). The 2-fold range in body length corresponds to a 16-fold increase in accumulation rate. In *Daphnia* (Fig. 7), accumulation rate again increases with body length at a very similar rate as for *Bosmina*. However, the proportional change is much smaller: the 2.5-fold range in body length corresponds to a 3.3-fold increase in accumulation rate. There is some suggestion in Fig. 7 that *Daphnia* with eggs have a higher accumulation rate. This may indicate that the accumulated ^{32}P for large, eggless *Daphnia* is lower because they have released labelled eggs during the experiment. This would create a slight underestimation of the true accumulation rate for large animals, and the rate at which accumulation increases with body size.

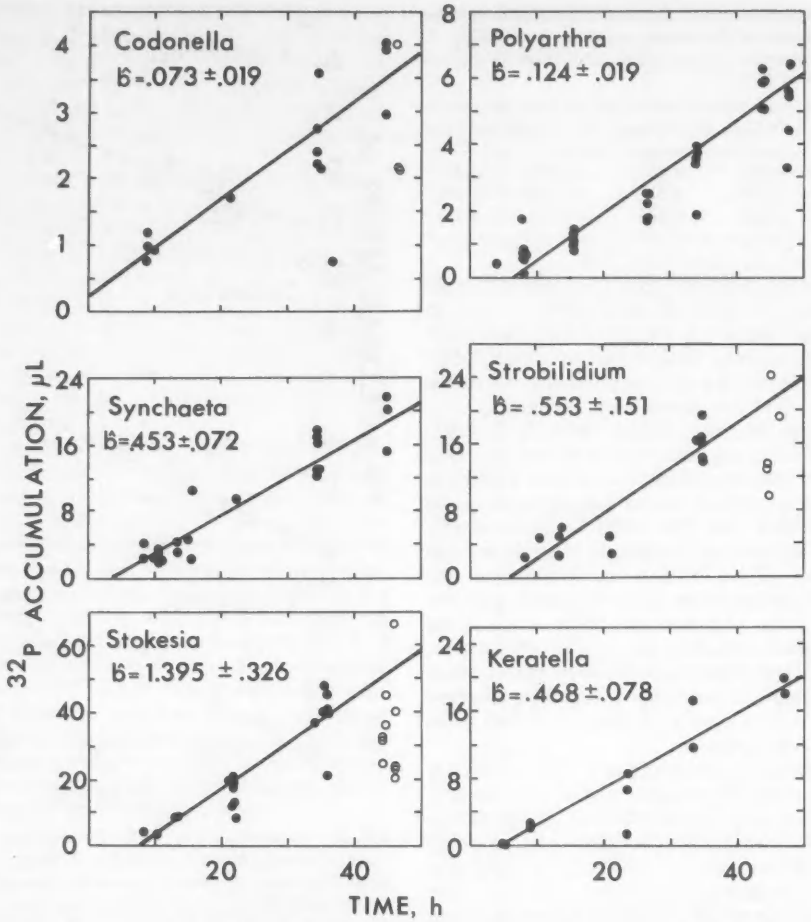

FIG. 4. Accumulation of ^{32}P by *Codonella cratera* (cruise 3), *Polyarthra vulgaris* (cruise 4), *Synchaeta* sp. (cruise 3), *Strobilidium velox* (cruise 3), *Stokesia vernalis* (cruise 3), and *Keratella cochlearis* (cruise 1). Slopes and 95% confidence limits are given. Open circles represent points not included in the regressions.

Phosphorus Release

Phosphorus release measurements presented some difficulties, especially for smaller species (Fig. 8). Release rates were extremely variable, and log transformation was required prior to parametric analyses. For each species, amounts of ^{32}P released during 10-min intervals were examined using two-way analyses of variance, treating individuals as replicates and time as a fixed effect. In most cases (*Synchaeta*, *Strombidium*, *Keratella*, *Strobilidium*, and *Daphnia*) there were significant differences among individuals in excretion rates ($P(F) \leq 0.05$). In only one case (*Strombidium*) was there a significant effect due to time. This was because the first 10-min period had a higher release rate (least significant difference test). I elected to use all release estimates for all species, rather than treat *Strombidium* differently. This means that there may be a systematic bias towards underestimating release rate for small species, such as *Strombidium*. This would result from a shorter residence time for ingested phosphorus.

Larger species, such as *Daphnia*, may tend to have more constant release rates, but they are still quite variable, as shown on a finer time scale by Scavia and McFarland (1982).

The relationship between the release rate of individuals and body size was investigated for *Bosmina*, *Daphnia*, and *Diacyclops*. *Bosmina* excretion tended to increase with body size (Fig. 9), but the description of this relationship is complicated by the tendency for these Cladocera to become trapped in the surface film. Two individuals were trapped repeatedly and were found afterwards to have released relatively little ^{32}P. These were omitted from the fitting of the regression line. Again, a linear fit appeared appropriate.

The same relationship is also shown for *Daphnia* in Fig. 9. A curvilinear regression was used to describe it, but note that the curvilinear fit is not significantly better than a linear one ($P(F) = 0.157$). Phosphorus release also increased with body size for *Diacyclops*.

Body Size and Phosphorus Flux

A major aim of this study was to examine the relationship

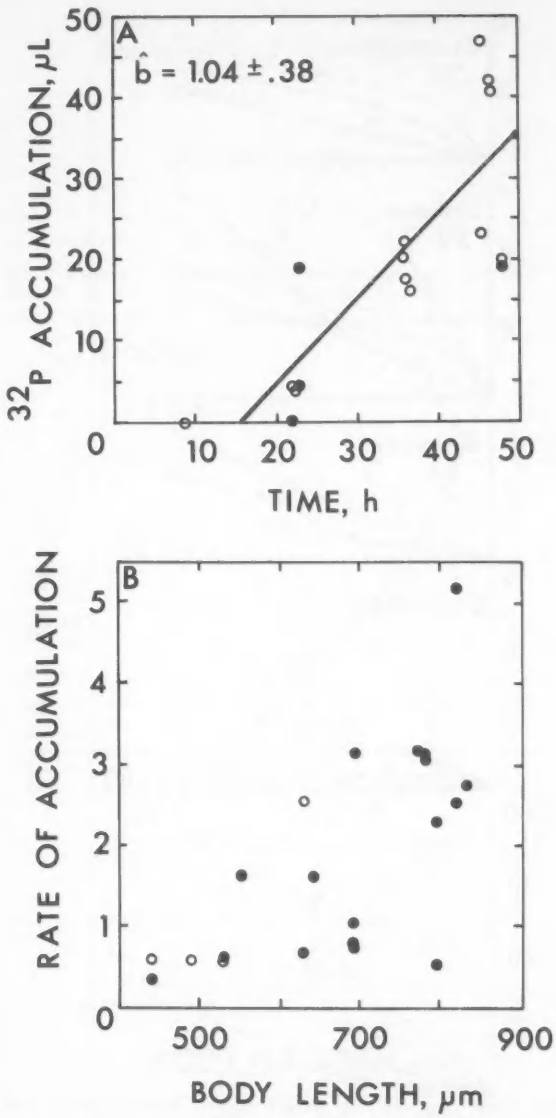

FIG. 5. (A) Accumulation of ^{32}P by cyclopoid copepods, all or mostly *Diacyclops bicuspidatus thomasi*. Open circles represent naupliii; solid circles represent copepodids. Data were collected on cruise 3. (B) Rate of ^{32}P accumulation, calculated for copepodids and adults, plotted against body length. Solid circles represent cruise 4; open circles represent cruise 3.

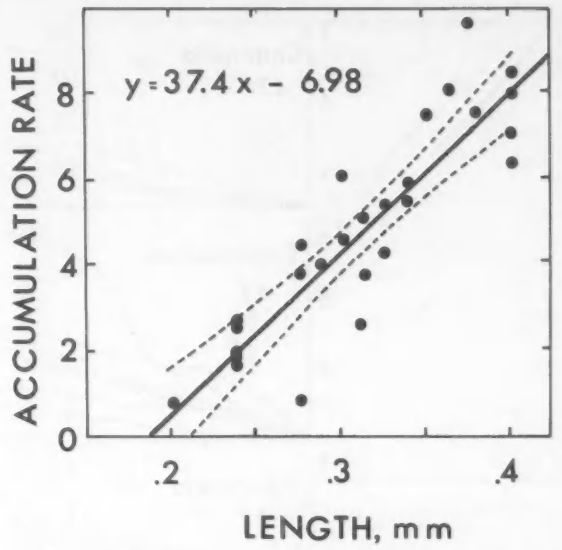

FIG. 6. Relationship between ^{32}P accumulation rate and body length for *Bosmina longirostris* on cruise 4. The least squares regression is shown, with 95% confidence limits for y. Accumulation rate is in $\mu L \cdot h^{-1}$.

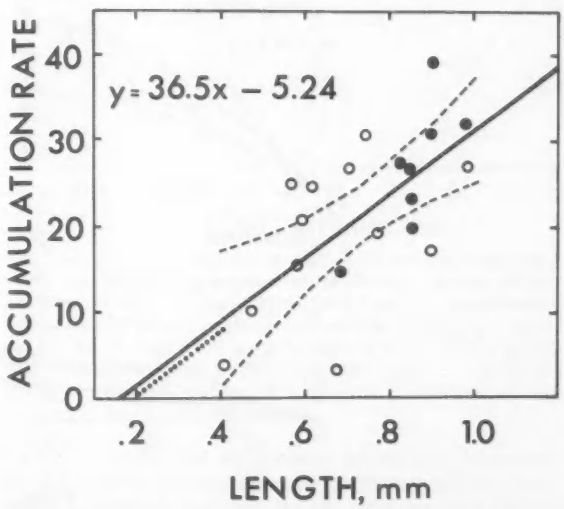

FIG. 7. Relationship between ^{32}P accumulation rate and body length for *Daphnia retrocurva*, with a least squares regression line and 95% confidence limits for y. Data were collected on cruise 5. Dotted line indicates the regression line for *Bosmina* from Fig. 6; open circles represent values for animals without eggs. Accumulation rate is in $\mu L \cdot h^{-1}$.

between body size and phosphorus flux. Although mass may be the best measure of body size, the techniques for measuring the mass of large and small zooplanktons are different, and so I have used length as a common measure.

The zooplankton species considered above range in length from 36 µm (average length of *Codonella*) to 983 µm (maximum observed for *Daphnia*), a 27-fold range. *Diacyclops* was excluded because its food was not properly labelled, and therefore, uptake rates are probably underestimated. Phosphorus uptake rate increases with body length (Fig. 10A), although not as rapidly as body volume or mass. A slope of 2 most closely approximates the data and is the expected result. Empirically derived relationships between metabolic parameters and body mass are usually power functions with exponents of $\frac{3}{4}$–$\frac{2}{3}$ (see Fenchel 1974; Gunther 1975). This corresponds to a slope of 2–2.25 in Fig. 10A, assuming mass is proportional to the cube of length. It is generally believed that filtering rate increases

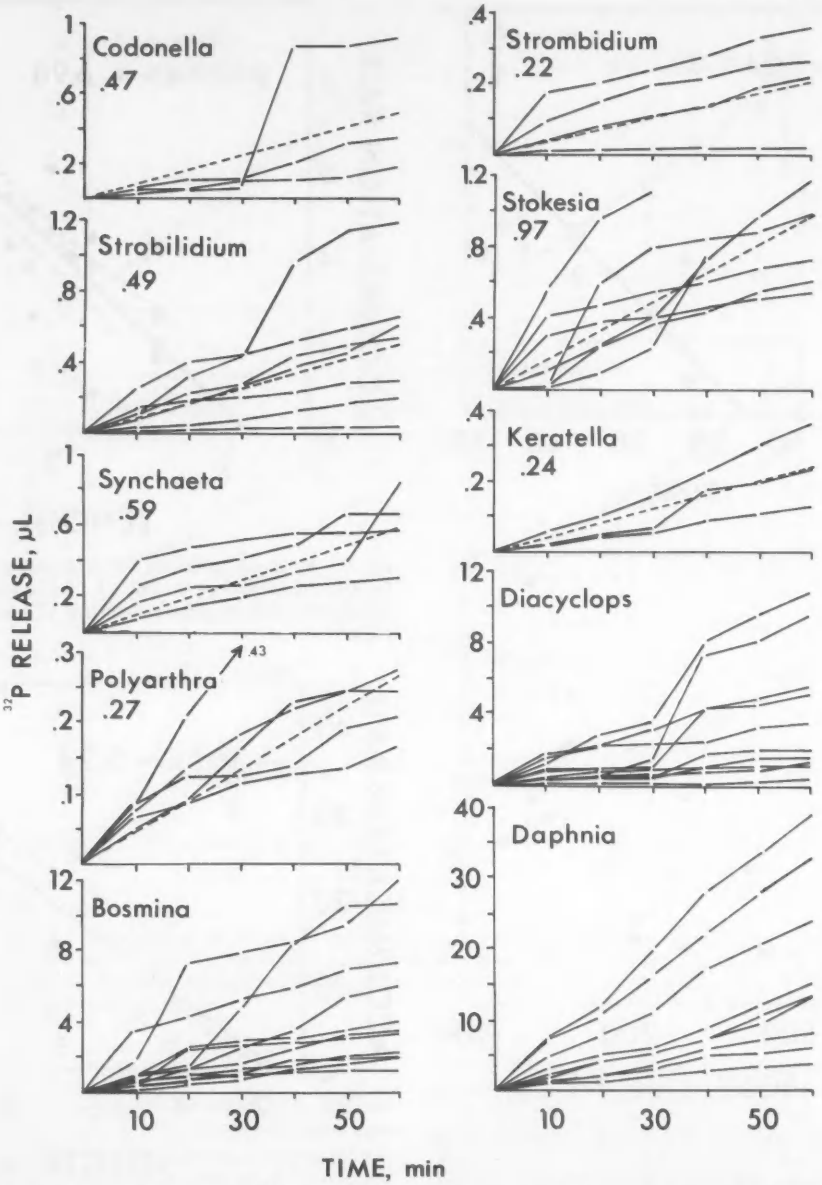

FIG. 8. Cumulative amount of ^{32}P released versus time by individuals of 10 zooplankton species. Dates and stations are the same as for Fig. 4–7, except for *Strombidium* (cruise 3), *Keratella* (cruises 2 and 3), and *Diacyclops* (cruises 2 and 3). Broken lines indicate mean rates for ciliates and rotifers.

with length in zooplankton with an exponent greater than 2, while respiration rate increases with an exponent less than 2 (see Hall et al. 1976 for a review). Peters and Rigler (1973) reported an exponent for *Daphnia* phosphorus excretion per unit weight of −0.38. Again assuming mass proportional to the cube of length, this would correspond to a slope of 1.86 in Fig. 10. It should be noted, however, that respiration and nutrient release were directly related to mass estimates in one recent study (Blažka et al. 1982), although over a smaller size range.

In summary, the relationship between phosphorus uptake and body length fits our current understanding of the allometry of metabolism and body size in general and of the feeding and excretion of zooplankton in particular. To put this allometry in perspective, we can look at the range in weight-specific metabolism illustrated in Fig. 10A. Over the 27-fold range in length, there is probably a 27^3- or 20 000-fold increase in mass, but only a 200-fold increase in phosphorus uptake for a 100-fold range in metabolism per unit mass. This is not due to a systematic bias resulting from small animals feeding on small prey items of higher specific activity; the ciliates *Strobilidium*

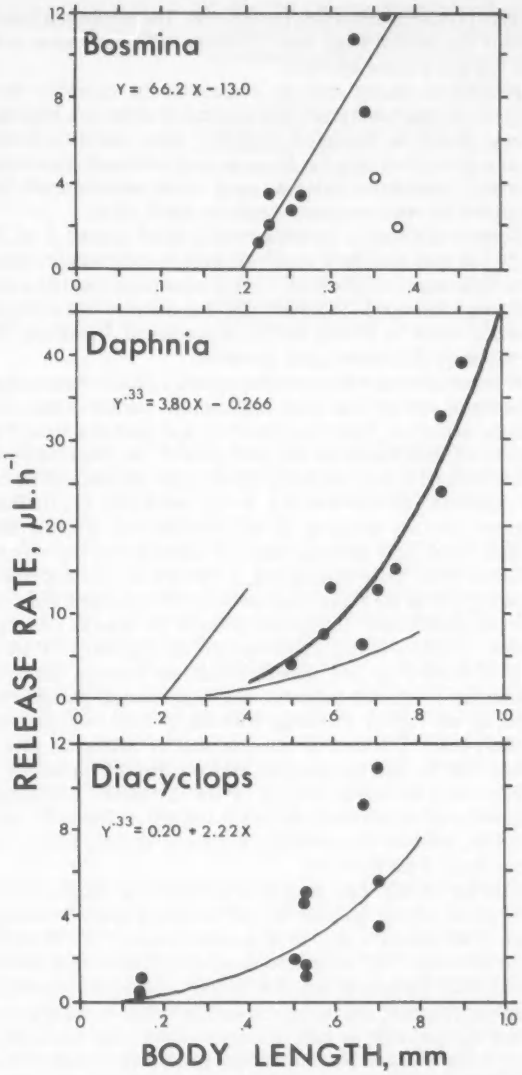

FIG. 9. Relationship between release rate and body length for *Bosmina*, *Daphnia*, and *Diacyclops*. The middle panel for *Daphnia* shows the other two regression lines for comparison. Open circles indicate points not included in the analysis.

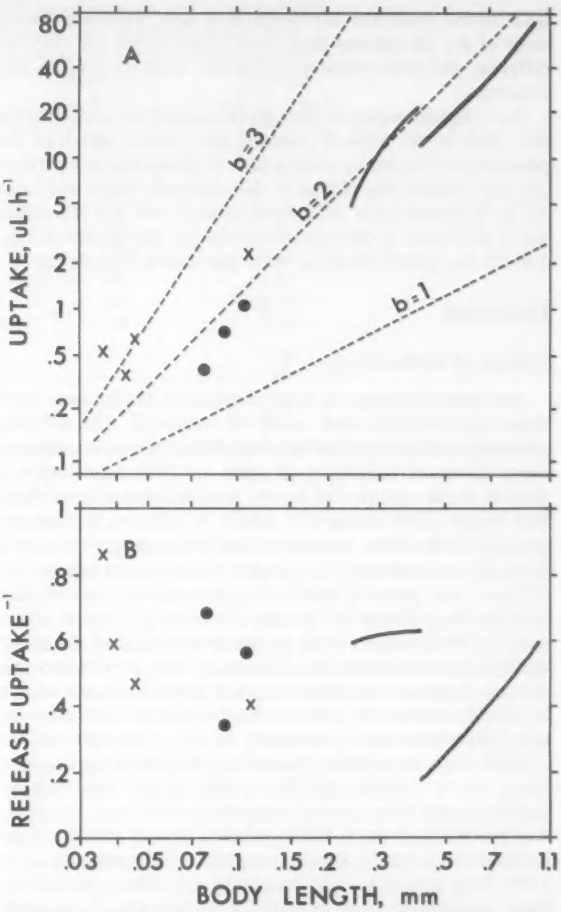

FIG. 10. (A) Double logarithmic plot of phosphorus uptake rate versus body length for nine species of zooplankton. Crosses, circles, and lines represent ciliates, rotifers, and Cladocera, respectively. Broken lines are shown for reference and indicate slopes of 1, 2, and 3. (B) Release of phosphorus as a proportion of uptake plotted against body length for the same species. From left to right: *C. cratera*, *S. viride*, *S. velox*, *P. vulgaris*, *K. cochlearis*, *Synchaeta* sp., *S. vernalis*, *B. longirostris*, *D. retrocurva*.

and *Stokesia* both feed on large algae, especially diatoms usually considered too large for Cladocera. The range in weight-specific metabolism is much greater than could be expected from differences in specific activity of food.

A problem related to the above is whether small zooplankton release a greater proportion of their phosphorus uptake. Release as a proportion of uptake ranges from 18 to 87% but is not markedly different between large and small species (Fig. 10B). The high value for *Codonella* (87%) is doubtful, as it is largely based on one large release event by one of three animals examined (Fig. 8). Calow (1977) reviewed conversion efficiencies in heterotrophic organisms based on mass, carbon, or energy and concluded that they range from 35 to 50% for metazoa and up to 60% for protozoa. Interspecific differences are related to feeding modes and the partitioning of assimilated energy according to energetic strategy, rather than size (Welch 1968). There is little reason to expect an interspecific correlation between body size and the proportion of ingested phosphorus released, and none is evident here.

There are size-dependent differences in phosphorus release per unit uptake within species (Fig. 10B). Conversion efficiencies generally decline with age, and therefore size, in metazoa (Calow 1977). Paloheimo et al. (1982) found accumulation efficiencies for *Daphnia pulex* to vary from 30 to 71% (therefore release per unit uptake 29–70%) with food concentration and body size, while Schindler (1968) found assimilitation efficiencies to decrease with body size for *D. magna*, as our data for *D. retrocurva* suggest. However, their method of calculating efficiencies is different from this paper, being based on mass or energy rather than phosphorus, and uses a single food rather

than natural plankton. My result, that large *Daphnia* release more of the phosphorus they ingest, may reflect that they eat different, and more frequently refractory, food items than small *Daphnia*.

An important aspect of phosphorus release not addressed by this study is the form of released phosphorus. Much of the phosphorus released by zooplankton is phosphate, but particulate and organic phosphorus is also released (Peters and Lean 1973; Taylor and Lean 1981; Madeira et al. 1982). It is possible that higher rates of phosphorus release per unit uptake in Fig. 10B are for species releasing more particulate phosphorus.

Discussion

Critique of Methodology

The methods used to measure phosphorus flux are new, have some shortcomings, and could be improved. The obvious advantage of these methods is that different species, ranging down in size to the limits of what can be handled under a dissecting microscope, can be studied simultaneously in whole lake water. Delicate species, such as ciliates, present no particular difficulties, and all measures are in comparable units. It should be emphasized that uptake rates measured here will be different, and probably lower, than conventional filtering rate estimates (e.g. Haney 1971) using labelled food. Similar values would be obtained only if the zooplankter in question filtered all the labelled components of the lake water, which will seldom be the case. Although the results provided by these methods are not as directly related to zooplankton feeding per se, they are appropriate to question concerning the flux of phosphorus.

An obvious assumption of the method for estimating accumulation rate is that the specific activity of the food quickly stabilizes and then remains relatively constant. The time required to label the food is reflected in the positive time-intercept in the relationship of accumulated versus time (Fig. 3, 4, 5). This is rapid for phytoplankton under the conditions of these experiments, but would not be at other seasons or locations. Special difficulties arise for predators whose food is slowly labelled, but these may not be insurmountable. They could, for example, be added to lake water long after the label was added.

It is also likely that even for species grazing on algae and bacteria there are differences in the specific activity of food items. For example, 0.2- to 1.0-μm particles are more strongly labelled than 1- to 12-μm particles (Fig. 2; Table 2). This can be altered experimentally (Taylor and Lean 1981). In Lake Ontario, when phosphorus demand is low, specific activity is most uniform. When phosphorus demand is high, the bias towards small cells in ^{32}P labelling can be eliminated by adding carrier ^{31}P-phosphate with the label (D. Lean, N.W.R.I., Burlington, Ont., pers. comm.).

Accumulation rates for ciliates are complicated by the process of binary fission. Shortening incubation times is a partial solution. Alternatively, collecting many more data points might allow the accumulation rate and the fission rate to be estimated from the pattern of ^{32}P accumulation versus time.

Rotifers and cladocerans present a slightly different problem; they may shed labelled eggs or neonates during the experiment. Again, the underestimation of accumulation through this error will increase with incubation time. Incubation times should be less than is required to manufacture and release reproductive products, so products released during the incubation will contain primarily unlabelled phosphorus. The incubation times used in the present study were probably sufficiently short that this was not a major problem.

Phosphorus release rates are plagued by the variability that exists at the individual level, and uncertainty about how long the release should be measured. Probably, rates would be better measured over less than 1 h. If one was not interested in the time course of phosphorus release, many more animals could be measured for one time interval with an equal effort.

Because of effects of containment, the small volume (1 mL) I employed may also be a source of error in estimating release rates from larger zooplankton. Larger containers could be used and then subsampled. Alternatively, the contents of the larger container could be filtered and the filter counted. Dissolved ^{32}P would be quickly taken up by particles.

A major difference between the method I used for measuring phosphorus release and other radioisotope studies is that the specific activity of the whole plankton was used as a basis for translating disintegrations into phosphorus. The usual method of translation has been to divide DPM by the specific activity of the organism's phosphorus (e.g. Peters and Rigler 1973). This assumes uniform labelling of the zooplankton, whereas the method used here assumes uniform labelling of the whole plankton. The latter assumption is preferable, because zooplankton will be the slowest component of the plankton to reach isotopic equilibrium. Except for perhaps the smaller ciliates, most zooplankton in this study were still accumulating ^{32}P after 48 h of incubation and therefore had not attained isotopic equilibrium. This also indicates that some internal phosphorus pools do not rapidly exchange with the medium, and so it is unlikely that ^{32}P release is being diluted by unlabelled phosphate. That is, internal phosphorus pools that did not become labelled must not be exchanging ^{31}P for ^{32}P. Most ^{32}P released is probably associated with the processing of labelled food, and therefore, released phosphorus has a similar specific activity to phosphorus in the food.

Lehman (1980) has criticized radioisotope measures of phosphorus release because isotope exchange could be taking place in the animal's gut, so a measured release of ^{32}P could occur without a "net" release of phosphorus. But this release of phosphorus (if it occurs) is still within any reasonable definition of nutrient release, and the corresponding influx of phosphorus across the gut must be part of accumulation (and ingestion). Phosphorus release is some fraction of phosphorus uptake; there is no "net release" for zooplankton.

Zooplankton Phosphorus Flux and Phytoplankton

The measured fluxes of phosphorus through zooplankton presented above can be put in context by comparing them with the total amount of phosphorus and reactive phosphate and the orthophosphate turnover time. These comparisons are only rough approximations, but do illustrate the potential significance of zooplankton phosphorus release.

Single zooplankton species at their maximum abundance could process up to $5\ mL \cdot L^{-1} \cdot h^{-1}$ or $0.5\% \cdot h^{-1}$ of the total phosphorus (Table 3); if all of these species were abundant at the same time and depth (which they were not), they might process $1.0\% \cdot h^{-1}$ or $24\% \cdot d^{-1}$ of the total phosphorus. This agrees with independent measures of community filtering rate, made on the same cruises, of $5–20\% \cdot d^{-1}$ (H. J. Fricker, N.W.R.I., Burlington, Ont., pers. comm.). The measured flux of orthophosphate corresponds to turnover times of 9–50 min (Table 1).

TABLE 3. Observed flux of phosphorus into different zooplankton species, their approximate maximum abundance in Lake Ontario epilimnion, and the product or total flux.

Species	Uptake ($\mu L \cdot h^{-1}$)		Abundance (number$\cdot L^{-1}$)	Total flux ($mL \cdot L^{-1} \cdot h^{-1}$)
Codonella cratera	0.54		1000	0.54
Strombidium ciride	0.40		3000	1.20
Strobilidium relox	1.04		500	0.52
Stokesia vernalis	2.37		200	0.47
Synchaeta sp.	1.04		100	0.10
Keratella cochlearis	0.71		500	0.36
Polyarthra vulgaris	0.39		500	0.19
Bosmina longirostris	11.1	(300 μm)	500	5.55
Daphnia retrocurva	33.8	(700 μm)	60	2.03
Sum				10.96

Using the ratio of soluble reactive to total phosphorus, we can calculate that the total phosphorus pool is replaced by this flux every 5–24 h or 4–20%$\cdot h^{-1}$. The flux of orthophosphate into zooplankton is apparently smaller.

More relevant to the role of zooplankton excretion to nutrient uptake by phytoplankton is a comparison of measured orthophosphate turnover times and the flux from zooplankton. A flux of 0.5%$\cdot h^{-1}$ into zooplankton might correspond to 0.25%$\cdot h^{-1}$ from zooplankton (assuming assimilation = 0.5 uptake, and all is available as PO_4). At SRP = 7% TP, this would replace the orthophosphate pool every 140–28 h. Again, the flux from zooplankton is small compared with the total orthophosphate flux. This is reflected in the observation that orthophosphate turnover time is not immediately affected by removing zooplankton (Lean and Charlton 1976; D. Hamilton, University of Waterloo, pers. comm.; unpublished observations). Inclusion of microzooplankton in phosphorus regeneration measurements, although they obviously play a major role, does not appear to alter the observation that the supply of phosphate from zooplankton does not appear sufficient to supply the needs of phytoplankton (Lean and Charlton 1976). However, some authors emphasize that the flux is sufficient to replace the SRP pool in a reasonably short period of time (e.g. Korstad 1983).

Lehman (1980) argued that the flux between phytoplankton and the water, as measured by $^{32}PO_4$ uptake, cannot be compared with zooplankton excretion, as the latter is a net flux. To compare zooplankton excretion with phytoplankton net uptake, as Lehman suggested, we should compare zooplankton phosphorus release with total phosphorus. A release of 0.25%$\cdot h^{-1}$ (see above) or 6.0%$\cdot d^{-1}$ is lower than expected for phytoplankton growth. From another direction, grazing of 20%$\cdot d^{-1}$ might regenerate 10%$\cdot d^{-1}$ of the total phosphorus, if 50% taken up is released. We should expect zooplankton to release enough of the nutrients they ingest to partly, but not entirely, allow for algal growth to replace those losses, as a model of Lake Ontario phosphorus cycling clearly indicates (Scavia 1979).

Shifts in Zooplankton Size-Distribution

In a stratified epilimnion where phosphorus limits phytoplankton biomass, and phytoplankton losses to zooplankton grazing are balanced by growth, zooplankton nutrient release could account for a substantial part of the net flux of phosphorus into phytoplankton; if they failed to assimilate phosphorus at all, but converted it to a useable form during gut passage, they would supply enough to balance grazing. To the extent that phosphorus uptake by zooplankton is converted to zooplankton biomass, released in a refractory form, or exported to the hypolimnion, zooplankton release will fail to balance phytoplankton requirements. Given this model of zooplankton–phytoplankton interaction, and the data above on zooplankton body size and phosphorus flux, how might shifting zooplankton size-distribution affect phytoplankton biomass?

The two major inferences that can be made from Fig. 10 are that small zooplankton would not release a significantly greater proportion of the phosphorus they ingest while grazing and that the standing stock of small zooplankton required to graze a given phytoplankton production would be much smaller than if the same production were grazed by large zooplankton. The replacement of large by small zooplankton would result in a much smaller zooplankton phosphorus sink.

The amount of phosphorus sequestered by zooplankton is variable; Prepas and Vickery (1984) reported that 3–31% of total phosphorus consists of particles greater than 250 μm, mostly zooplankton, in 17 Alberta lakes. Particles greater than 216 μm contained about 12% of the total phosphorus in the euphotic zone of Bob Lake, Ontario (Prepas and Rigler 1982). Nakashima and Leggett (1980) reported that zooplankton accounted for 22–40% of the seston phosphorus for Lake Memphramagog, Quebec. Prepas and Vickery (1984) also reported that particulate phosphorus greater than 250 μm accounted for a significant proportion of the residual variation in the relationship between spring total phosphorus and summer chlorophyll in their lake. The fraction of total phosphorus greater than 250 μm was also negatively related to total chlorophyll; greener lakes had relatively less zooplankton biomass.

Zooplankton also transfer epilimnetic phosphorus to higher trophic levels. Nakashima and Leggett (1980) found that in Lake Memphramagog, fish could contain as much phosphorus as the seston. In Lake Wingra, Wisconsin, bluegills contained 70% of the total phosphorus (Kitchell et al. 1975). A size-shift from a high biomass of large zooplankton to low biomass of small zooplankton would reduce the flux of phosphorus to this potentially large sink.

The rate at which phosphorus is lost from lake epilimnia by sedimentation is also variable; retention coefficients for lakes vary from near 0 to near 100%$\cdot yr^{-1}$. Although much of this variation is related to flushing time (e.g. Larsen and Mercier 1976), it is likely that sedimentation rates from epilimnia vary with the nature of the zooplankton community. Zooplankton faecal pellets are an important component of sedimentation in

Lake Michigan (Ferrante and Ptak 1978). Faecal pellets quickly decompose as they sink, and large pellets, which are produced by some large zooplankton, are more likely to escape the epilimnion before being stripped of their nutrients. Large zooplankton are also more likely to sink out of the epilimnion on dying, or swim out daily. If zooplankton migrate diurnally, and have higher feeding rates higher in the water column, their net effect will be to transport nutrients downward. Bürgi et al. (1979) examined the effect of removing large zooplankton on sedimentation rates in limnocorrals. Absence of large zooplankton increased sedimentation rate as it increased algal standing crop (chlorophyll concentration). Sedimentation rate as a fraction of carbon accumulation declined with increased biomass of large zooplankton, but its phosphorus to carbon ratio was higher.

In summary, the data presented here indicate that a major difference between large and small zooplankton with respect to nutrient fluxes is activity per unit biomass. Zooplankton communities dominated by large zooplankton should therefore have a relatively large zooplankton standing crop. Other properties of large zooplankton might reinforce the effect on phytoplankton biomass of a larger zooplankton phosphorus sink by further reducing nutrients available to phytoplankton. The hypothesis that high nutrient release rates for small zooplankton would generate a greater supply of nutrients for phytoplankton is untenable, because it assumes that zooplankton biomass would remain equal as size-distribution changes. This study suggests the testable hypothesis that zooplankton communities dominated by small species should have lower biomass, and more of the total phosphorus present as algae.

Acknowledgments

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NOTES

Uptake Efficiency of the Gills of English Sole (*Parophrys vetulus*) for Four Phthalate Esters

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Boese, B. L. 1984. Uptake efficiency of the gills of English sole (*Parophrys vetulus*) for four phthalate esters. Can. J. Fish. Aquat. Sci. 41: 1713–1718.

Uptake efficiencies of the gills of English sole (*Parophrys vetulus*) for four homologous phthalic acid esters were directly measured and compared with octanol–water partition coefficients (K_{ow}). Test compounds were diethyl phthalate (DEP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), and di-*n*-octyl phthalate (DOC). Mean uptake efficiencies in a single pass through the gills were 11% for DEP (log K_{ow} = 3.2), 42% for BBP (log K_{ow} = 5.8), 3% for DEHP (log K_{ow} = 8.7), and 12% for DOC (log K_{ow} = 9.2). Uptake efficiencies for DEP and BBP were inversely correlated with weight-specific ventilation volume and did not correlate with aqueous concentration. Uptake efficiencies for DOC and DEHP were uncorrelated with any measured parameter. Solubilities of phthalic acid esters were measured by generator column. DEP and BBP had aqueous solubilities similar to published values, but DEHP was 1/700 and DOC was 1/3000 below published solubility values. DOC and DEHP used to determine uptake efficiencies were most likely in colloidal or micellar form, which may be less bioavailable than soluble forms of the compound; this would reduce the apparent uptake efficiency, regardless of K_{ow} value.

On a mesuré directement l'efficacité de l'absorption de quatre esters phtaliques homologues chez une espèce de sole (*Parophrys vetulus*) et l'on a comparé les résultats aux coefficients de partage octanol–eau (K_{oe}). Les quatres composés étaient : le phtalate de diéthyle (DEP), le phtalate de butyle et de benzyle (BBP), le phtalate de bis(2-éthylhexyle) (DEHP) et le phtalate de di-*n*-octyle (DOC). Les efficacités moyennes d'absorption en un seul passage sur les branchies étaient de 11 % pour le DEP (log K_{oe} = 3,2), 42 % pour le BBP (log K_{oe} = 5,8), 3 % pour le DEHP (log K_{oe} = 8,7) et 12 % pour le DOC (log K_{oe} = 9,2). L'efficacité d'absorption pour le DEP et le BBP était en corrélation inverse avec la ventilation spécifique (v/m) et sans rapport avec la concentration dans l'eau. Pour le DEHP et le DOC il n'y avait corrélation avec aucun des paramètres mesurés. La solubilité des esters phtaliques a été mesurée sur colonne génératrice. Le DEP et le BBP avaient des solubilités dans l'eau conformes aux valeurs publiées, mais le DEHP et le DOC n'avaient, respectivement, que 1/700 et 1/3000 des solubilités publiées. Dans le cas de ces deux derniers esters, nous avions probablement une forme colloïdale ou micellaire moins biodisponibles que les formes solubles ; cela réduirait donc l'efficacité apparente d'absorption, indépendamment de la valeur de K_{oe}.

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The uptake of numerous organic pollutants from water by fish has been correlated with the compounds' octanol–water partition coefficient (Neely et al. 1974; Lu and Metcalf 1975; Chiou et al. 1977). If pollutant uptake from water is dependent upon lipid and aqueous phase partitioning, then uptake efficiency should be related to the octanol–water partition coefficient (K_{ow}). This hypothesis was indirectly tested (Neely 1979) by deriving the uptake efficiencies of several organic compounds using a mathematical model developed by Norstrom et al. (1976) and regressing these predicted efficiencies against corresponding K_{ow} values. This suggested a linear relationship between uptake efficiencies and K_{ow} over a wide range of K_{ow} values (log 3–12).

The work reported here was designed to test this relationship by directly measuring the concentration of four phthalate esters of differing K_{ow} value in the inspiratory and expiratory flows of English sole (*Parophrys vetulus*). Additionally, the effects of phthalate concentration and ventilatory volume on gill uptake efficiency were measured.

Phthalate esters, used to impart flexibility to plastics, are a homologous group of compounds that vary greatly in K_{ow}. The U.S. Environmental Protection Agency has included phthalate

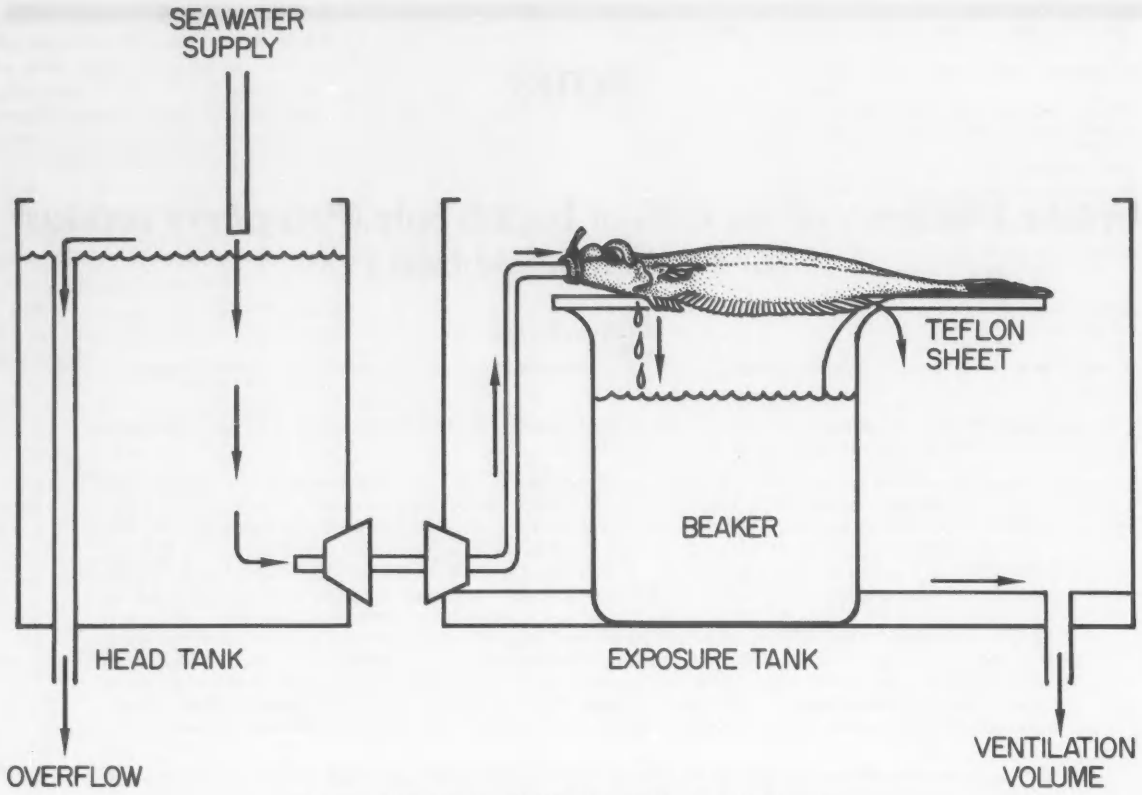

FIG. 1. Dosing apparatus used in uptake efficiency experiments.

esters as priority pollutants because of their high production volumes (Peakall 1975) and their worldwide detection as contaminants in soils, sediments, and animal tissues (Gledhill et al. 1980; Mayer et al. 1972; Ogner and Schnitzer 1970; Morris 1970).

Materials and Methods

Young-of-the-year English sole were collected by otter trawl from Yaquina Bay, OR, and maintained on a diet of Oregon Moist Pellets for at least 3 mo before experimentation. Holding aquaria (250 L) were supplied with aerated seawater (25–32‰, 12 ± 2°C) at a minimum flow rate of $20\,L \cdot h^{-1}$. Total fish biomass never exceeded $2\,g \cdot L^{-1} \cdot d^{-1}$ in any holding aquarium.

Uptake efficiency — The exposure apparatus (Fig. 1) consisted of four rectangular glass aquaria (75 × 200 × 175 mm) connected by Z-shaped glass cannulae (5 mm) to a single glass head tank (300 × 200 × 175 mm). Water level in the head tank was adjusted such that a column of seawater filled the cannula but did not flow out unless pulled by fish respiratory movements.

Fish were anesthetized (0.05% benzocaine) and immobilized by suturing to a 1.6-mm-thick teflon sheet (8 × 15 cm) from which a section had been removed immediately below the opercula. A 250-mL beaker positioned under the opercula served both the support the fish and to collect effluent water.

Glass cannulae in each of the three experimental aquaria were inserted into the mouth of the fish and secured in place with an 8-mm nylon cable tie. Care was taken to minimize injury to the fish and to allow free movement of the floor of the mouth. The body posterior to the gills was covered with moist gauze and the aquarium was covered with aluminum foil to reduce moisture loss. Sand-filtered, UV-sterilized seawater (25–32‰, 12 ± 0.5°C) was supplied to the head tank at $100\,mL \cdot min^{-1}$ for a 2-h recovery period. Water pulled from the head tank by the ventilatory movements of the fish passed through the gills and dripped from the opercula into a collection beaker, with individual fish controlling the ventilation volume. Following the recovery period, seawater in the head tank was replaced by seawater containing dissolved phthalate ester. This dosing solution was prepared by adding an appropriate amount of phthalate stock solution (2–20 mL) to 45 L of sterile seawater (25–32‰, 12°C), followed by vigorous stirring for at least 2 h. This provided a minimum dilution factor for the stock solutions of 1/2000. The phthalate esters tested were butyl benzyl (BBP), di-*n*-octyl (DOC), diethyl (DEP), and bis(2-ethylhezyl) (DEHP) phthalates. Phthalate stock solutions were prepared by dissolving 0.5 g of pure phthalate per litre of acetonitrile carrier. Pure phthalate solutions were obtained from Pfaltz and Bauer, Stamford, CT (BBP), Eastman, Rochester, NY (DEHP), and Chem. Services Inc., West Chester, PA (DOC and DEHP).

Phthalate solutions were supplied to the head tank ($100\,mL \cdot min^{-1}$) via a constant head siphon (Marriotte bottle) for a 3-h exposure. Flow rate of dosing solution was always in excess of that utilized by the fish. In each test, three fish were exposed to a single concentration of phthalate ester. Four to five

TABLE 1. Uptake efficiency exposure conditions and physiological parameters (V/wt = weight-specific ventilation volume).

Phthalate	Concn. range ($\mu g \cdot L^{-1}$)	Mean (SE) fish wt. (g)	V/wt (range) ($L \cdot h^{-1} \cdot g^{-1}$)	Mean (SE) ventilation rate ($beats \cdot min^{-1}$)	n
DEP	30–200	32.7(1.6)	0.009–0.037	32(1)	34
BBP	20–250	28.3(1.0)	0.014–0.065	35(2)	28
DEHP	25–300	26.7(1.3)	0.010–0.060	32(1)	12
DOC	30–40	38.5(2.7)	0.010–0.037	34(2)	13

tests (12–15 fish) were independently performed on DEP, BBP, and DEHP at each of three concentration ranges to test for the effect of phthalate concentration on uptake efficiency. Concentration ranges tested were low (20–35 $\mu g \cdot L^{-1}$), high (190–300 $\mu g \cdot L^{-1}$) and intermediate (95–125 $\mu g \cdot L^{-1}$). For DOC, four replicate tests were made within a single concentration range (30–40 $\mu g \cdot L^{-1}$).

In the control aquarium the cannula height was adjusted so that water dripped into the collection beaker at approximately the same rate as the average ventilation volume of an experimental fish (10–20 $mL \cdot min^{-1}$). Control aquarium water samples were used for inspiratory phthalate determinations. Overflow from the beakers in experimental aquaria was collected to determine ventilation volume. Ventilation rates were determined by counting opercular movements for five 1-min periods at convenient intervals during the experiment. These rates were compared with rates of nonexperimental fish in holding aquaria.

Phthalate analysis — Independent, triplicate, 100-mL samples were taken from expiratory water and control aquaria (inspiratory water) for phthalate concentration measurements beginning 2 h after the addition of phthalate dosing solution. Absolute methanol (10 mL) was added to each sample before phthalate extraction. Phthalate uptake efficiencies were determined by (inspired concn. − expired concn.)/inspired concn. Phthalates were extracted from the water–methanol mixture by alkyl bonded phase columns (C-2 Bond-Elut Analytichem International Inc., Harbor City, CA) following a method used to extract pesticides from water (Andrews and Good 1982). The column retained the phthalates as well as other lipophilic compounds, which were then eluted off with an organic solvent (2 mL acetonitrile). The eluate was analyzed by a Hewlett-Packard 5880 A gas chromatograph (3-μL spitless injections) with a fused silica column (15 m, 0.24 mm ID, liquid-film thickness of 1.0 μm) and flame ionization. The column temperature was elevated in two stages: $30° \cdot min^{-1}$ from 70 to 220°C followed by $15° \cdot min^{-1}$ to 320°C. Recoveries of phthalate esters from control aquaria were 90% (DEP), 72% (BBP), 56% (DEHP), and 63% (DOC) of nominal dose, with a coefficient of variation of 8–15%. These recoveries reflect both analytical error and loss within the system (e.g. adsorption to glass surfaces). Concentrations reported in the text were not corrected for recovery.

Water solubilities — Results of the uptake efficiency experiments brought into question the accuracy of published solubility values for DEHP and DOC. Solubilities of the four phthalate esters were measured using a generator column similar to that described by May (1979). Uncontaminated, filter-sterilized (0.45 μm) seawater was pumped through a sand column containing an excess (1 g phthalate · 100 g $sand^{-1}$) of the compound of interest. The extraction methodology for these samples was changed from that reported above in an attempt to improve recoveries. Phthalates were extracted using C-18 Bond-Elut columns followed by elution with ethylacetate and analyzed by electron capture capillary gas chromatography. Recoveries of phthalate-spiked 100-mL water samples (20–85 $\mu g \cdot L^{-1}$) ranged from 72- for DEHP to 100% for DEP, BBP, and DOC, with a coefficient of variation of 4–8%.

Statistical analysis — Associations between uptake efficiency and physiological–chemical parameters were determined by product-moment correlations at $p < 0.05$ significant level. Regression analysis was by model II regression (Bartlett 1949) using a computer program described by Davies (1971).

Results

Experimental exposure conditions and fish physiology data are summarized in Table 1. Uptake efficiencies for BBP and DEP were inversely correlated with weight-specific ventilation volume ($p < 0.01$) and uncorrelated with fish weight and phthalate exposure concentration ($p > 0.5$). DEHP and DOC uptake efficiencies were not significantly correlated with any measured parameter. Mean ventilation rates did not vary significantly between experiments ($p > 0.5$), nor were experimental ventilation rates significantly different from fish in holding aquaria (mean = 31 $movements \cdot min^{-1}$, $se = 1$, $n = 25$).

As weight-specific ventilation volume increases, uptake efficiency of BBP and DEHP (Fig. 2) decreases. Slopes of the two regression lines are not significantly different ($p > 0.5$) but intercepts and means are different ($p < 0.01$).

Phthalate seawater solubilities (25‰, 12°C) determined by generator column were similar to published values for BBP and DEP, but were 7000 and 3000 times below published values for DEHP and DOC, respectively (Table 2).

Mean uptake efficiency of fish gills increased approximately fourfold as log K_{ow} increased from 3.2 to 5.8 (DEP to BBP) (Table 2). There was no clear relationship between log K_{ow} and uptake efficiencies for DEHP and DOC ($k_{ow} > 8.7$).

Discussion

Uptake efficiency methodology — The dosing apparatus allowed for simultaneous direct measurements of uptake efficiency on three experimental fish with minimal body surface contact with expired water. Ventilation rates of cannulated fish did not differ significantly from uncannulated controls in holding aquaria; however, the wide range of weight-specific ventilation volumes (0.010–0.065 $L \cdot h^{-1} \cdot g^{-1}$) may partially

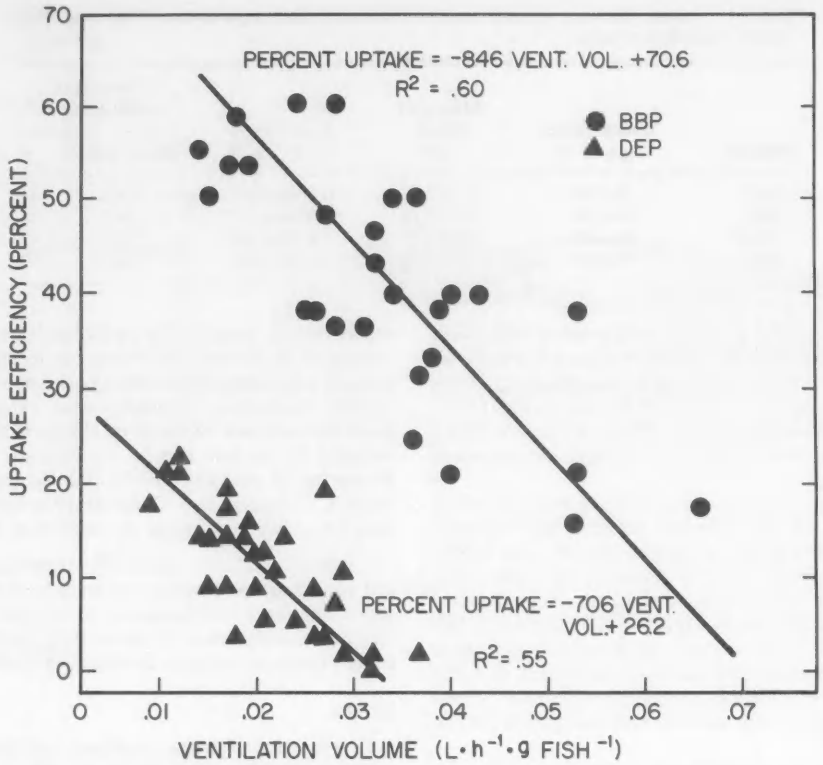

FIG. 2. Uptake efficiency of English sole gills compared with weight-specific ventilation volume. ●, BBP; ▲, DEP.

TABLE 2. Uptake efficiency of English sole gills for four phthalate esters.

Phthalate	log K_{ow}[a]	Solubility ($\mu g \cdot L^{-1}$)[b]	Solubility ($\mu g \cdot L^{-1}$)[a]	Mean uptake (%), $\bar{x}$ (SE)
DEP	3.2	720 000	1 000 000	11.3(1.1)
BBP	5.8	1 900	2 900	42.2(2.5)
DEHP	8.7	0.6	400	2.9(1.2)[c]
DOC	9.2	1.0	3 000	12.4(2.1)[c]

[a]Published solubility values (freshwater at 23–32°C) and K_{ow} values (U.S. Environmental Protection Agency 1980).

[b]Solubility determined in seawater (25‰, 12°C) by the column method described by May (1979).

[c]Uptake efficiencies determined at water concentrations in excess of solubilities determined by generator column.

result from slight hydrostatic head differences between fish. Preliminary experiments showed that fish could control the flux of water through their gills even if the hydrostatic head was elevated or depressed slightly below fish level. Fish could be maintained for up to 7 d in the experimental apparatus; however, abrasion of oral tissue by the cannula limits long-term use.

Elevating fish out of the water was necessary, since the English sole secreted copious amounts of mucous which clogged bonded phase chromatography columns. The presence of mucous did not alter column extraction efficiency; however, the volume of sample that could pass through the column was reduced. Gill mucous may hinder organic uptake, and stress (e.g. chemical, handling) is likely to enhance mucous production. Although gill mucous is a likely source of experimental error, no gross differences in mucous production were noted across the four phthalates tested or among concentrations of any single phthalate.

Effect of ventilation volume — The results showing that DEP and BBP uptake efficiency was inversely correlated to ventilation volume and independent of concentration are consistent with published results for endrin uptake efficiency in brook trout (*Salvelins fortinoulis*) (McKim and Goeden 1982). These reductions in phthalate uptake efficiencies with increasing gill ventilation are likely caused by the same factors that reduce oxygen uptake efficiency at increased ventilation volumes. As gill ventilation increases, diffusion dead space (distance between lamellae) and anatomical dead space (water passing between ends of filaments) increase, and progressively more of the inspired water is shunted away from absorbtive (respiratory) surfaces (Randall 1970). At high ventilation volumes, inspired water may be in contact with the gills for an insufficient time for dissolved oxygen to reach equilibrium with blood (Randall 1970).

Solubility — Large differences were found between published and generator column derived solubility values for DEHP and DOC (Table 2). Although the published values were determined in freshwater and at higher temperatures than used in this study, the disparity between DEHP and DOC solubility

values probably cannot be explained by temperature and salinity differences alone.

Published solubility values for phthalate esters were determined by mixing (shaking or stirring) an excess of phthalate esters with water. For polynuclear aromatic hydrocarbons (PAHs) these mechanical techniques have produced supersaturated solutions with the "dissolved" PAH existing in micellar of colloidal state with water (Peake and Hodgson 1976). Use of generator columns in solubility determinations appears to reduce formation of micelles and colloids (May 1979), yielding lower, and probably more realistic, solubility estimates for compounds with low aqueous solubility.

Effect of k_{ow} — Uptake efficiency of English sole gills was greater for BBP than DEP. This result was expected if uptake efficiency of organic compounds increases with increasing lipid solubility and K_{ow}. If this relationship has general application, an explanation is needed for why DOC and DEHP did not follow the rule. For polychlorinated biphenyls (PCBs), the relationship between bioconcentration factors (BCFs) and K_{ow} also is not linear; with decreasing BCFs noted with $\log K_{ow} > 6$ (Sugiura et al. 1978; Shaw and Connell 1982). Suggested explanations for these results are that highly lipophilic PCBs translocate slowly to storage sites within the organism, thus slowing uptake (Sugiura et al. 1978), or that steric effects due to the location and number of chlorines on the PCB molecule hinder uptake (Shaw and Connell 1982). A simpler explanation for the data presented in this paper on the uptake efficiencies for phthalate esters is that exposure concentrations of DOC and DEHP were in excess of their water solubility limits (Table 2), resulting in gills being exposed to micellar or colloidal suspensions of the compounds. This may have reduced uptake efficiencies relative to soluble forms of the same compound due to surface area effects.

In bioconcentration experiments, exceeding solubility limits would extend the time required to reach equilibrium. Mayer (1976), for example, found that as the aqueous concentration of DEHP was increased, the BCF into tissues of fathead minnows (*Pimephales promelas*) decreased. Mayer suggested that this might be the result of increased depuration through induction of detoxification enzymes at the highest exposure concentrations. A more likely explanation is that at the DEHP concentrations used by Mayer (2–62 $\mu g \cdot L^{-1}$), a portion of the compound was not in solution and was not equally available for uptake. Reductions in uptake efficiency with exposure concentrations beyond solubility limits would lengthen the time required for body burden to reach equilibrium, leading to an underestimation of BCF.

When supersaturated solutions are used in bioconcentration experiments, multiphasic uptake might occur due to differential bioavailability of the soluble portion over colloid fractions. Mayer's (1976) data on DEHP uptake into fathead minnows appear to be biphasic with a rapid initial uptake (1–7 d) followed by slower uptake (14–56 d). Since BCF determinations are often made from experiments of limited duration, biphasic uptake could lead to underestimation of BCF.

Implications — In nature, organic compounds exist in soluble, colloidal, and particulate phases. Uptake efficiencies and rates of waterborne organics into tissues of aquatic organisms are likely to be dependent upon these phases. For example, sorption of pesticides to suspended sediments reduces BCFs (Eaton et al. 1983) and acute toxicity (Brungs and Bailey 1967) in fathead minnows. Since compounds in solution probably are taken up more readily through respiratory surfaces than are compounds in suspension, an organism may reach equilibrium more rapidly with the soluble phase than with the colloidal or particulate phase. In some previous bioconcentration studies using hydrophobic compounds, organisms may have been exposed to multiphasic dosing solutions. In these experiments equilibrium body burdens may not have been reached and BCFs were probably underestimated. In short-term experiments, BCFs can only be determined accurately if the exposure concentration of a compound in all its physical–chemical phases is known. Further research is needed to define and quantify these states in nature and to determine how these states affect uptake.

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BOOK REVIEWS/COMPTES RENDUS

Scombrids of the World. *Bruce B. Collette and Cornelia E. Nauen. FAO Species Catalogue. FAO Fisheries Synopsis No. 125. Vol. 2. Rome, Italy. 137 p. 1983.*

This is the second in a series of species catalogues covering a group of marine food species on a worldwide basis. The first in the series, volume 1, published in 1980, was entitled *Shrimps and Prawns of the World*.

This catalogue provides indepth treatment of the 49 known species of scombrids. A review of the significant morphological, anatomical, and osteological features of the Scombridae precedes a detailed and well-illustrated key to all species. Each species is then treated in detail providing the most recent information on synonymy, diagnostic features, world distribution, general biology, local names, and information on parasites and toxicity when known. A section entitled *Interest to Fisheries* provides pertinent information on existing fisheries, gear employed, landings, and historical background.

The treatment for the 49 species is uneven because of the disparity in available information. Research and study has been concentrated on the commercially important species.

Scombrids of the World is well-illustrated with excellent black and white illustrations in the introductory sections, the keys, and "the species accounts."

Anyone concerned with scombrid fishes — administrator, biologist, technician, or sportsman — will find this a most useful text. FAO is to be commended for providing funds for this project and for having Dr. Walter Fischer as editor. The authors are to be congratulated for the excellent quality of this review. Unfortunately, the price of the volume is not noted anywhere.

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(R584)

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Variability of chlorophyll *a* off southwestern Nova Scotia in late fall and its relationship to water column stability — **R. O. Fournier, R. Ernst, N. B. Hargreaves, M. Van Det, and D. Douglas**

Toxicity of nitrite to channel catfish (*Ictalurus punctatus*), talapia (*Talapia aurea*), and largemouth bass (*Micropterus salmoides*): evidence for a nitrite exclusion mechanism — **R. M. Palachek and J. R. Tomasso**

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Size at maturity of Northwest Atlantic redfishes (*Sebastes*) — **I-H. Ni and E. J. Sandeman**

Wind resuspension of sediments in a prairie lake — **G. L. Carper and R. W. Bachmann**

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Ambient pH and calcium concentration as modifiers of growth and calcium dynamics of book trout, *Salvelinus fontinalis* — **D. W. Rodgers**

Postglacial dispersal of freshwater fishes in the Québec peninsula — **P. Legendre and V. Legendre**

Influence of reservoir transit on riverine algal transport and abundance — **D. M. Søballe and R. W. Bachmann**

Cross correlations between reconstructed ocean abundances of Bristal Bay and British Columbia sockeye salmon (*Oncorhynchus nerka*) — **R. M. Peterman and F. Y. C. Wong**

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Density-dependent growth in early ocean life of sockeye salmon (*Oncorhynchus nerka*) **R. M. Peterman**

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Diel vertical migration and feeding behaviour of a demersal hydromedusan (*Polyorchis penincillatus*) — **S. A. Arkett**

Role of fish in regulation of plant and animal communities in eutrophic ponds — **C. N. Spences and D. L. King**

Predation by rock crabs (*Cancer irroratus*) on diseased sea urchins (*Strongylocentrotus droenbachiensis*) in Nova Scotia — **R. Scheibling**

Mortality estimation: biased results from the unbiased ages — **J. Barlow**

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